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TRANSMITTAL OF VERIFIED TRANSLATION

Attached herewith is a verified English language translation of the priority documents listed below, which we previously filed in this case:

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VERIFICATION OF TRANSLATION

I, Masaaki IWABUCHI, 35-7-201, Nozawa 1-chome, Setagaya-ku, Tokyo 154-0003 Japan, state the following:

I am fluent in both the English and Japanese languages and capable of translating documents from one into the other of these languages.

The attached document is a true and accurate English translation to the best of my knowledge and belief of:

Japanese Patent Application No. 1999-157111

I state that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Signature : Masaaki Iwabuchi

Masaaki IWABUCHI

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[Title of the Invention] SCREENING METHOD USING CD100

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[Document] SPECIFICATION

[Title of the Invention] Screening Method Using CD100

[Claims]

[Claim 1] A screening method for compounds or salts thereof that change the binding property between CD100 or salts thereof and receptor thereof characterized by using CD100 or salts thereof as a ligand.

[Claim 2] A screening kit for compounds or salts thereof that change the binding property between CD100 or salts thereof and receptor thereof characterized by using CD100 or salts thereof as a ligand.

[Claim 3] The screening method of Claim 1 or the screening kit of Claim 2 wherein the receptor is CD72 or salt thereof.

[Claim 4] Compounds or salts thereof that change the binding property between CD100 or salts thereof and CD72 or salts thereof obtained by using the screening method of Claim 1 or the screening kit of Claim 2.

[Claim 5] The compounds or salts thereof of Claim 4 that promote or inhibit the activity of CD100 or salts thereof.

[Claim 6] Pharmaceuticals that contain the compounds or salts thereof of Claim 4.

[Claim 7] The pharmaceuticals of Claim 6 that are antibody production inducers or, agents for prevention or treatment of diseases caused by abnormal antibody production.

[Claim 8] The pharmaceuticals of Claim 7 wherein the disease caused by abnormal antibody production is an allergy or autoimmune disease.

[Claim 9] The screening method of Claim 1 characterized by adding CD100 or salt thereof, or CD100 or salt thereof and test compound to cells expressing CD72 and measuring changes in the antibody level produced or secreted by the expressing cells.

[Detailed description of the Invention]

[0001]

[Technical Field to which the Invention pertains]

The present invention relates to a screening method for compounds or salts thereof that are useful as antibody production inducers or as agents for the prevention or treatment of diseases caused by abnormal antibody production characterized by using CD100 (Proceedings of the National Academy of Sciences of the USA (Proc. Natl. Acad. Sci. USA), Vol. 93 (1996), pp. 11780-11785, etc.) or salts thereof and receptors thereof, for example, CD72 (Journal of Immunology (J. Immunol.), Vol. 149 (1992), pp. 880-886, etc.).

[0002]

[Prior Art]

B cells can produce any one of five types of antibodies comprising IgM, IgD, IgG, IgA, and IgE. IgM is produced first in terms of gene structure when the B cells differentiate and first encounter an antigen in vivo. However, the physiological function of IgM is weaker than that of the other classes of antibodies. When the stimulation by the same antigen continues, the gene is changed and the classes of antibodies other than IgM are produced, thereby actualizing stronger physiological function. This change in immunoglobulin from IgM to other classes is called class switch.

CD40 is a membrane glycoprotein expressed on B cells. It reacts, for example, with CD40L expressed on activated T cells. There is known to be no antibody production, class switch, or vaccine effect in CD40-deficient mice. CD40 is a molecule essential for the antibody production function of B cells. Stimulation of B-cells by CD40 inhibits B cell death by anti-IgM antibody. Stimulation of the B cells by CD40 also induces production of various antibody classes, including IgM. However, it is still unclear how these B cell responses are induced.

[0003]

[Problems to be solved by the Invention]

Controlling B cell death and class switch should make it possible to regulate B cell antibody production.

Epidemic diseases could be treated effectively if it were possible to rapidly raise the post-vaccination

antibody titer to epidemic diseases such as colds and influenza where rapid antibody production is required. However, such a therapeutic method does not currently exist. It would also be an effective means of treating so-called allergies and autoimmune diseases if it were possible to specifically lower the abnormal antibodies in diseases caused by abnormal antibody production such as atopic asthma, atopic dermatitis, rheumatoid arthritis, and allergic rhinitis. However, such as a therapeutic method does not currently exist.

[0004]

[Means for solving problems]

The inventors isolated and acquired the gene induced by CD40 and clarified that this molecule is CD100. Furthermore, they elucidated that death of B cell by anti-IgM antibody can be avoided when CD100 forms complexes by binding to CD72 on B cells that have been stimulated by activating factors such as CD40, IL-4 or LPS and that CD100 plays an extremely important role in inducing class switch. When complexes are formed by CD100 binding to CD72 on B cells that have been stimulated by activating factors such as CD40, IL-4 or LPS, class switch is triggered in the B-cells and specific high-affinity antibodies are strongly induced in vivo. These facts demonstrate that substances that induce binding of CD72 and CD100, substances that substitute CD100 and bind to CD72 in place of CD100, substances that heighten binding to CD72 by partial modification of the CD100 molecule, and CD100 itself are effective therapeutic methods for rapidly raising the post-vaccination antibody titer against epidemic diseases, for example, such as colds and influenza.

CD100 also serves as an immunopotentiator against cancer and infections. Substances that inhibit the binding of CD72 and CD100 also serve as effective methods of treating diseases caused by abnormal antibody production, such as atopic asthma, atopic dermatitis, rheumatoid arthritis, and allergic rhinitis, as they are

expected to inhibit only antibody production of activated B cells.

[0005]

That is, the present invention provides:

(1) A screening method for compounds or salts thereof that change the binding property between CD100 or salts thereof and receptor thereof characterized by using CD100 or salts thereof as a ligand,

(2) A screening kit for compounds or salts thereof that change the binding property between CD100 or salts thereof and receptor thereof characterized by using CD100 or salts thereof as a ligand,

(3) The screening method of (1) above or the screening kit of (2) above wherein the receptor is CD72 or salt thereof,

(4) Compounds or salts thereof that change the binding property between CD100 or salts thereof and CD72 or salts thereof obtained by using the screening method of (1) above or the screen kit of (2) above,

(5) The compounds or salts thereof of (4) above that promote or inhibit the activity of CD100 or salts thereof,

(6) Pharmaceuticals that contain the compounds or salts thereof of (4) above,

(7) The pharmaceuticals of (6) above that are antibody production inducers or agents to prevent or treat diseases caused by abnormal antibody production,

(8) The pharmaceuticals of (7) above wherein the diseases caused by abnormal antibody production are allergies or autoimmune diseases,

(9) The screening method of (1) above characterized by adding CD100 or salt thereof or CD100 or salt thereof and test compound to cells expressing CD72 and measuring the changes in the antibody levels produced or secreted by the expressing cells,

[0006]

Specific examples of CD100 in the present invention include not only publicly known CD100 and salts thereof (Proc. Natl. Acad. Sci USA, Vol. 93 (1996), pp. 11780-

11785; Journal of Biological Chemistry, Vol. 271 (1996), pp. 33376-33381), but also

(10) Polypeptides characterized by containing the same or substantially the same amino acid sequence as the amino acid sequences shown by SEQ ID NO: 1 or SEQ ID NO: 3 (hereinafter referred to as CD100) or salts thereof or

(11) The CD100 or salts thereof of (10) wherein the polypeptide is a protein that contains an amino acid sequence with from 1 to 30, preferably 1 to 10, amino acids deleted from the amino acid sequence shown by SEQ ID NO: 1 or by SEQ ID NO: 3, amino acid sequence with from 1 to 30, preferably 1 to 10, amino acids added (or inserted) in the amino acid sequence shown by SEQ ID NO: 1 or SEQ ID NO: 3, or amino acid sequence with from 1 to 30, preferably 1 to 10, amino acids substituted by other amino acids in the amino acid sequence shown by SEQ ID NO: 1 or SEQ ID NO: 3.

Specific examples of CD72 in the present invention include known CD72 and salts thereof [The Journal of Immunology, Vol. 144, pp. 4870-4877 (1990); The Journal of Immunology, Vol. 149, pp. 880-886 (1992)]. Mouse CD72 also includes allotypes such as Lyb-$2^{a.1}$, Lyb-$2^{a.2}$, Lyb-2^{b}, and Lyb-2^{c} described in The Journal of Immunology, Vol. 149, pp. 880-886 (1992). Further examples of CD72 include:

(12) Polypeptides characterized by containing the same or substantially the same amino acid sequence as the amino acid sequences shown by SEQ ID NO: 5 or SEQ ID NO: 7 (hereinafter referred to as CD72) or salts thereof or

(13) The CD72 or salts thereof of (12) above wherein the polypeptide is a protein that contains an amino acid sequence with from 1 to 10, preferably 1 to 5, amino acids deleted from the amino acid sequence shown by SEQ ID NO: 5 or SEQ ID NO: 7, amino acid sequence with from 1 to 10, preferably 1 to 5, amino acids have been added (or inserted) in the amino acid sequence shown by SEQ ID NO: 5 or SEQ ID NO: 7, or amino acid sequence with from 1 to 10, preferably 1 to 5, amino acids have been substituted

by other amino acids in the amino acid sequence shown by SEQ ID NO: 5 or SEQ ID NO: 7.

[0007]

The term "substantially the same" in the specification means that the activity of the polypeptide, etc., e.g., binding activity of the ligand (CD100) and receptor (CD72), physiological properties, etc., are substantially the same. The substitution, deletion, addition or insertion of amino acids may not greatly change the physiological characteristics or chemical properties of the polypeptide. In such cases, the polypeptide that has undergone the substitution, deletion, addition or insertion (so-called mutated CD100, mutated CD72, etc.) is said to be substantially the same as that which has not undergone substitution, deletion, addition or insertion. Essentially the same substitutes of the amino acids in said amino acid sequence can be selected from among other amino acids in the class to which this amino acid belongs. Examples of nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine. Examples of polar (neutral) amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. Examples of amino acids with a positive electrical charge (basic) include arginine, lysine, and histidine. Examples of amino acids with a negative electrical charge (acidic) include aspartic acid and glutamic acid.

[0008]

[Modes for carrying out the Invention]

Methods for manufacturing the CD100 and CD72 used in the present invention will be explained in more detail below.

Examples of the CD100 and CD72 used in the present invention include polypeptides derived from the tissues (e.g., pituitary, pancreas, brain, kidneys, liver, gonads, thyroid, gallbladder, bone marrow, adrenals, skin, muscles, lungs, digestive tract, blood vessels, heart) or cells of humans, warm-blooded animals (e.g., guinea pigs,

rats, mice, pigs, sheep, cows, monkeys) and fish. The CD100 may be any as long as it is a polypeptide that contains the same or substantially the same amino acid sequence as the amino acid sequence shown by SEQ ID NO: 1 or SEQ ID NO: 3. The CD72 may be any as long as it is a polypeptide that contains the same or substantially the same amino acid sequence as the amino acid sequence shown by SEQ ID NO: 5 or SEQ ID NO: 7. Examples of substantially the same amino acid sequence as the amino acid sequence shown by SEQ ID NOs: 1, 3, 5 or 7 include amino acid sequences with at least approximately 70%, preferably at least approximately 80%, more preferably at least approximately 90%, even more preferably at least approximately 95%, homology with the amino acid sequence shown by SEQ ID NOs: 1, 3, 5 or 7. For example, examples of CD72 include polypeptides with substantially the same activity as polypeptides that contain the amino acid sequence shown by SEQ ID NO: 5 or SEQ ID NO: 7 in addition to polypeptides that contain the amino acid sequence shown by SEQ ID NO: 5 or SEQ ID NO: 7. Examples of substantially the same activity include ligand binding activity, signal transduction activity, and antibody production capacity. "Essentially the same activity" means that the two substances have substantially the same properties in ligand binding activity, for example. Therefore, intensity or weakness such as the intensity of ligand binding activity and quantitative criteria such as the molecular weight of the polypeptide may differ. Examples of CD100 include polypeptides with substantially the same activity as polypeptides that contain the amino acid sequence shown by SEQ ID NO: 1 or SEQ ID NO: 3 in addition to polypeptides that contain the amino acid sequence shown by SEQ ID NO: 1 or SEQ ID NO: 3. Examples of substantially the same activity include receptor binding activity and antibody production activity. "Essentially the same activity" means that the two substances have substantially the same properties in receptor binding activity, for example. Therefore, intensity or weakness such as the intensity of receptor

binding activity and quantitative factors such as the molecular weight of the polypeptide may differ.

[0009]

In the CD72 and CD100 in this specification, the left end is the N terminal (amino terminal) and the right end is the C terminal (carboxyl terminal) according to peptide designation custom. For example, the C terminal of polypeptides that contain an amino acid sequence shown by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, or SEQ ID NO: 7 is usually a carboxyl group (-COOH) or carboxylate ($-COO^-$), but the C terminal may be an amide ($-CONH_2$) or ester (-COOR). Examples of R of the ester include the pivaloyloxymethyl groups commonly used as esters for oral use in addition to C_{1-6} alkyl groups such as methyl, ethyl, n-propyl, isopropyl, and n-butyl, C_{3-8} cycloalkyl groups such as cyclopentyl and cyclohexyl, C_{6-12} aryl groups such as phenyl and α-naphthyl, and C_{7-14} aralkyl groups such as phenyl-C_{1-2} alkyl such as benzyl, phenethyl, and benzhydryl or α-naphthyl-C_{1-2} alkyl such as α-naphthylmethyl.

Salts of physiologically acceptable bases (such as alkali metals, etc.) and acids (organic acids and inorganic acids) are used as the salts of CD72 and CD100 used in the present invention. However, physiologically acceptable acid addition salts are especially preferred. Salts with inorganic acids (e.g., hydrochloric acid, phosphoric acid, hydrobromic acid, sulfuric acid), or salts with organic acids (e.g., acetic acid, formic acid, propionic acid, fumaric acid, maleic acid, succinic acid, tartaric acid, citric acid, maleic acid, oxalic acid, benzoic acid, methanesulfonic acid, benzenesulfonic acid) are used as such salts.

The CD72 and CD100 used in the present invention can be manufactured by methods that accord with the publicly known methods [The Journal of Immunology, Vol. 144, pp. 4870-4877 (1990); The Journal of Immunology, Vol. 149, pp. 880-886 (1992); Proc. Natl. Acad. Sci. USA, Vol. 93 (1996), pp. 11780-11785; Journal of Biological Chemistry, Vol. 271 (1996), pp. 33376-33381], i.e., by purifying the

polypeptide from the tissues or cells of humans or warm-blooded animals. They can also be manufactured in accordance with the polypeptide synthesis techniques described below. They can also be manufactured by culturing transformants that contain DNA encoding the polypeptide as described below.

When they are manufactured from the tissues or cells of human, warm-blooded animals, fish, etc., the tissues or cells of the human, warm-blooded animal, or fish, etc., are homogenized, and then extraction by acids or an organic solvent, etc are performed. The polypeptide can then be purified and isolated by submitting said extract to a combination of salting out, dialysis, gel filtration, and chromatography such as reverse-phase chromatography, ion-exchange chromatography, and affinity chromatography.

[0010]

As mentioned above, the CD72 and CD100 used in the present invention can be manufactured according to publicly known peptide synthesis techniques or by cleaving a polypeptide that contains the polypeptide by a suitable peptidase. The peptide synthesis technique may be either a solid-phase or liquid-phase synthesis technique. That is, the target peptide can be manufactured by condensing partial peptides or amino acids capable of constructing the polypeptide and residual parts, and removing the protective groups when the product has protective groups. Methods (1) - (5) listed below are examples of publicly known methods for condensation and removal of protective groups.

(1) M. Bodanszky and M. A. Ondetti, *Peptide Synthesis*, Interscience Publishers, New York (1966).

(2) Schroeder and Luebke, *The Peptide*, Academic Press, New York (1965).

(3) N. Ezumiya et al., *Peptide Synthesis Fundamentals and Experiments*, Maruzen (1975).

(4) H. Yashima and T. Sakakibara, Biochemical experimentation lectures 1, Protein Chemistry IV, 205 (1977).

(5) H. Yashima (editor), Continued drug development, Vol. 14, Peptide Synthesis, Hirokawa Shoten.

The polypeptide (CD72 or CD100) can also be purified and isolated after reaction by a conventional purification method, i.e., a combination of solvent extraction, evaporation, column chromatography, liquid chromatography, and recrystallization. When the polypeptide obtained by the aforementioned methods is a free compound, it can be converted into a suitable salt by publicly known methods. Conversely, when it is obtained in the form of salt, it can be converted into a free compound by publicly known methods.

[0011]

Amide forms of CD72 and CD100 can use commercial resins for peptide synthesis suited to amide formation. Examples of such resins include chloromethyl resin, hydroxymethyl resin, benzhydrylamine resin, aminomethyl resin, 4-benzyloxybenzyl alcohol resin, 4-methylbenzhydrylamine resin, PAM resin, 4-hydroxymethyl-methylphenylacetamide methyl resin, polyacrylamide resin, 4-(2',4'-dimethoxyphenyl-hydroxymethyl)phenoxy resin, and 4-(2',4'-dimethoxyphenyl-Fmoc aminoethyl)phenoxy resin. Using such resins, α-amino groups and amino acids with suitably protected side chain functional groups are condensed on the resin through various known condensation methods in accordance with the sequence of the target peptide. The protective groups are removed simultaneously with cutting the peptide from the resin after the end of the reaction and the target polypeptide is acquired by an intramolecular disulfide bond formation reaction in a highly dilute solvent if necessary.

For condensation of the protected amino acids described above, a variety of activation reagents for peptide synthesis may be used, but carbodiimides are particularly preferably employed. Examples of such carbodiimides include DCC, N,N'-diisopropylcarbodiimide, N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide, etc. For activation by these reagents, the protected amino acids in combination with a racemization inhibitor (e.g., HOBt,

etc.) are added directly to the resin, or the protected amino acids are previously activated in the form of symmetric acid anhydrides or HOBt esters, followed by adding the thus activated protected amino acids to the resin. Solvents suitable for use to activate the protected amino acids or condense with the resin may be chosen from solvents that are known to be usable for peptide condensation reactions. Examples of such solvents are acid amides such as N,N-dimethylformamide, N,N-dimethylacetamide, N-methylpyrrolidone and others, halogenized hydrocarbons such as methylene chloride, chloroform and others, alcohols such as trifluoroethanol, sulfoxides such as dimethylsulfoxide, amines such as pyridine, ethers such as dioxane and tetrahydrofuran, nitriles such as acetonitrile and propionitrile, esters such as methyl acetate and ethyl acetate, or appropriate mixtures of these solvents. The reaction temperature is appropriately chosen from the range known to be applicable to peptide bond-forming reactions and is usually selected in the range of approximately -20°C to 50°C. The activated amino acid derivatives are used generally in an excess of 1.5 to 4 times. The condensation is examined using the ninhydrin reaction; when the condensation is insufficient, the condensation can be completed by repeating the condensation reaction without removal of the protecting groups. When the condensation is yet insufficient even after repeating the reaction, unreacted amino acids are acetylated with acetic anhydride or acetylimidazole to cancel any possible adverse affect on the subsequent reaction.

Examples of the protecting groups used to protect amino groups of the starting materials include Z, Boc, tertiary-penthyloxycarbonyl, isobornyloxycarbonyl, 4-methoxybenzyloxycarbonyl, Cl-Z, Br-Z, adamantyloxycarbonyl, trifluoroacetyl, phthaloyl, formyl, 2-nitrophenylsulphenyl, diphenylphosphinothioyl, Fmoc, etc. Examples of the protecting groups for carboxyl group include 2-adamantyl, 4-nitrobenzyl, 4-methoxybenzyl, 4-chlorobenzyl, phenacine groups, benzyloxycarbonyl

hydrazide, tertiary-butoxycarbonyl hydrazide, trityl hydrazide in addition to C_{1-6} alkyl groups, C_{3-8} cycloalkyl groups and C_{7-14} aralkyl groups stated above as R.

The hydroxyl group of serine and threonine can be protected by, for example, its esterification or etherification. Examples of groups appropriately used for the esterification include a lower alkanoyl group such as acetyl group, etc., an aroyl group such as benzoyl group, etc., and a group derived from carbon such as benzyloxycarbonyl group and ethoxycarbonyl group. Examples of the group appropriately used for the etherification include benzyl group, tetrahydropyranyl group, tertiary-butyl group, and the like.

Examples of groups for protecting the phenolic hydroxyl group of tyrosine include Bzl, Cl_2-Bzl, 2-nitrobenzyl, Br-Z, tertiary-butyl, etc.

Examples of groups used to protect the imidazole moiety of histidine include Tos, 4-methoxy-2,3,6-trimethylbenzenesulfonyl, DNP, benzyloxymethyl, Bum, Boc, Trt, Fmoc, etc.

Examples of the activated carboxyl groups in the starting material include the corresponding acid anhydrides, azides, activated esters (esters with alcohols (e.g., pentachlorophenol, 2,4,5-trichlorophenol, 2,4-dinitrophenol, cyanomethyl alcohol, p-nitrophenol, HONB, N-hydroxysuccimide, N-hydroxyphthalimide, HOBt)). As the activated form of the amino groups in the starting material, the corresponding phosphoric amides are employed.

[0012]

To eliminate (split off) the protecting groups, there are employed catalytic reduction in a hydrogen gas flow in the presence of a catalyst such as Pd-black or Pd-carbon; an acid treatment with anhydrous hydrogen fluoride, methanesulfonic acid, trifluoromethanesulfonic acid or trifluoroacetic acid, or a mixture solution of these acids; a treatment with a base such as diisopropylethylamine, triethylamine, piperidine or piperazine; and reduction with sodium in liquid ammonia.

The elimination of the protecting group by the acid treatment described above is carried out generally at a temperature of -20°C to 40°C. In the acid treatment, it is efficient to add a cation scavenger such as anisole, phenol, thioanisole, m-cresol, p-cresol, dimethylsulfide, 1,4-butanedithiol or 1,2-ethanedithiol. Furthermore, 2,4-dinitrophenyl group known as the protecting group for the imidazole of histidine is removed by a treatment with thiophenol. Formyl group used as the protecting group of the indole of tryptophan is eliminated by the aforesaid acid treatment in the presence of 1,2-ethanedithiol, 1,4-butanedithiol or the like, as well as by a treatment with an alkali such as a diluted sodium hydroxide solution and dilute ammonia.

Protection of functional groups that should not be involved in the reaction of the starting materials, protecting groups, elimination of the protecting groups and activation of functional groups involved in the reaction may be appropriately selected from publicly known groups and publicly known means.

In another method for obtaining the amides of CD72 and CD100, for example, the α-carboxyl group of the carboxy terminal amino acid is first protected by amidation; the peptide chain is then extended from the amino group side to a desired length. Thereafter, a protein in which only the protecting group of the N-terminal α-amino group has been eliminated from the peptide chain and a peptide (or amino acids) in which only the protecting group of the C-terminal carboxyl group has been eliminated are manufactured. The two peptides are condensed in a mixture of the solvents described above. The details of the condensation reaction are the same as described hereinabove. After the protected peptide obtained by the condensation is purified, all the protecting groups are eliminated by the method described above to give the desired crude polypeptide. This crude polypeptide is purified by various known purification means. Lyophilization of the major fraction gives the amide of the desired polypeptide.

When obtaining an ester form of CD72 or CD100, an ester form of the desired polypeptide can be obtained in the same way as the amide form of the polypeptide after producing an amino acid ester by condensing the α-carboxyl groups of the amino acid at a carboxyl terminal with the desired alcohol.

[0013]

The DNA encoding CD72 used in the present invention may be any of the DNA that contains DNA encoding receptor protein comprising the same or substantially the same amino acid sequence as that shown by SEQ ID NO: 5 or SEQ ID NO: 7. The DNA encoding CD72 used in the present invention may be any of the DNA that contains DNA encoding ligand protein comprising the same or substantially the same amino acid sequence as that shown by SEQ ID NO: 1 or SEQ ID NO: 3. It may be any of genomic DNA, genomic DNA library, cDNA derived from the aforementioned tissues or cells, cDNA library derived from the aforementioned tissues or cells, or synthetic DNA. The vectors used in the library may be any of bacteriophages, plasmids, cosmids, or phagemids. It can also be amplified directly by reverse transcriptase polymerase chain reaction (abbreviated hereinafter as RT-PCR) using an RNA fraction prepared from the aforementioned tissues or cells.

More specifically, (1) DNA that hybridizes with sequences that have DNA that contains DNA encoding a receptor protein with an amino acid sequence the same or substantially the same as the amino acid sequence shown by SEQ ID NO: 5 or SEQ ID NO: 7 (or SEQ ID NO: 1 or SEQ ID NO: 3) under stringent conditions and (2) DNA encoding a polypeptide that has the same amino acid sequence even though it does not form a hybrid with the sequence set forth in (1) and a sequence with DNA that contains DNA encoding a polypeptide that contains an amino acid sequence the same or substantially the same as the sequence shown by SEQ ID NO: 5 or SEQ ID NO: 7 (or SEQ ID NO: 1 or SEQ ID NO: 3) due to degeneration of the genetic code, etc. is used. Hybridization can be performed by or

in accordance with known methods. An example of the aforementioned stringent conditions is 42°C, 50% formamide, 4 × SSPE (1 × SSPE = 150 mM NaCl, 10 mM $NaH_2PO_4 \cdot H_2O$, 1 mM EDTA, pH 7.4), 5 × Denhardt solution, 0.1% SDS.

[0014]

The DNA encoding CD72 or CD100 used in the present invention can be manufactured as well by the following genetic engineering means.

Means of cloning DNA encoding CD72 or CD100 in its entirety include amplification of the target DNA from the aforementioned DNA library, etc., by known PCR using a synthetic DNA primer that has a partial base sequence of the polypeptide or hybridization of DNA incorporated in a suitable vector with one labeled using synthetic DNA or a DNA fragment that contains part or all of the polypeptide region. Hybridization is performed, for example, by the methods described in *Molecular Cloning* (2nd edition: J. Sambrook et al., Cold Spring Harbor Lab. Press, 1989). When using a commercial library, it is conducted by the method described in the attached instructions.

The cloned DNA encoding CD72 or CD100 used in the present invention can be used as is or digested by restriction enzymes with linkers added as desired, depending on the goal. Said DNA may have ATG as a translation initiation codon on the 5' terminal and TAA, TGA, or TAG as a translation stop codon on the 3' terminal. These translation initiation codons and translation stop codons can also be added using appropriate synthetic DNA adapters.

Expression vectors of the CD72 and CD100 used in the present invention can be produced, for example, by (a) cutting the target DNA fragment from the DNA encoding CD72 or CD100 used in the present invention and (b) ligating said DNA fragment downstream of the promoter in a suitable expression vector.

Plasmids derived from *Escherichia coli* (e.g., pBR322, pBR325, pUC12, pUC13, plasmids derived from *Bacillus subtilis* (e.g., pUB110, pTP5, pC194), plasmids derived

from yeast (e.g., pSH19, pSH15), bacteriophages such as λ phage, and animal viruses such as retrovirus, vaccinia virus, and baculovirus are used as vectors. Any promoter can be used as long as it is a suitable promoter that corresponds to the host used in gene expression.

[0015]

When the host in transformation is an animal cell, a promoter derived from SV40, a retrovirus promoter, metallothioneine promoter, heat shock promoter, cytomegalovirus promoter, SRα promoter, etc., can be utilized. When the host is an organism of the genus Escherichia, a trp promoter, T7 promoter, lac promoter, recA promoter, λPL promoter, lpp promoter, etc., is preferred. When the host is an organism of the genus Bacillus, an SPO1 promoter, SPO2 promoter, penP promoter, etc., is preferred. When the host is yeast, a PHO5 promoter, PGK promoter, GAP promoter, ADH1 promoter, GAL promoter, etc. is preferred. When the host is an insect cell, a polyhedrin promoter, P10 promoter, etc., is preferred.

In addition to the above, those that contain enhancers, splicing signals, poly A addition signals, selection markers, SV40 origin of replication (sometimes abbreviated hereinafter as SV40ori), etc., can be used for the expression vector. Examples of selection markers include a dihydrofolate reductase (sometimes abbreviated hereinafter as dhfr) gene [methotrexate (MTX) resistance], ampicillin resistance gene (sometimes abbreviated hereinafter as Amp^r), and neomycin resistance gene (sometimes abbreviated hereinafter as Neo, G418 resistance). When CHO ($dhfr^-$) cells are used and a DHFR gene used as the selection marker in particular, selection is possible even by thymidine-free medium.

The signal sequence joined to the host if necessary is added on the N terminal of the polypeptide or partial peptide thereof. A PhoA signal sequence, OmpA signal sequence, etc. can be used when the host is an organism of the genus Escherichia. A α-amylase signal sequence or subtilysin signal sequence, etc. can be used when the

host is an organism of the genus Bacillus. A mating factor α (MFα) signal sequence or invertase signal sequence, etc. can be used when the host is yeast. Insulin signal sequence, α-interferon signal sequence, or antibody molecule signal sequence, etc. can be used when the host is an animal cell.

Transformants can be produced using vectors that contain DNA encoding CD72 or CD100 constructed in this way.

[0016]

Organisms of the genus Escherichia, organisms of the genus Bacillus, yeasts, insects or insect cells, animal cells, etc., can be used as the host.

Escherichia coli K12·DH1 [Proc. Natl. Acad. Sci. USA, Vol. 60, 160 (1968)], JM103 [Nucleic Acids Research, Vol. 9, 309 (1981)], JA221 [Journal of Molecular Biology, Vol. 120, 517 (1978)], HB101 [Journal of Molecular Biology, Vol. 41, 459 (1969)], C600 [Genetics, Vol. 39, 440 (1954)], etc., are used as organisms of the genus Escherichia.

Bacillus subtilis MI114 [Gene, Vol. 24, 255 (1983)], 207-21 [Journal of Biochemistry, Vol. 95, 87 (1984)], etc., are used as organisms of the genus Bacillus, etc.

[0017]

Saccharomyces cerevisiae AH22, AH22R, NA87-11A, DKD-5D, 20B-12 are used as yeasts.

Silkworm larvae, for example, are used as insects [Maeda et al., Nature, Vol. 315, 592 (1985)].

As insect cells, for example a cell line from cabbage looper pupae (*Spodoptera frugiperda* cells; Sf cells), MG1 cells from the midgut of *Trichoplusia ni*, High Five™ cells from *Trichoplusia ni* eggs, cells from *Mamestra brassicae*, and cells from *Estigmena acrea* are used as insect cells when the virus is AcNPV. A silkworm cell line (*Bombyx mori* N; BmN cells), etc. is used when the virus is BmNPV. Sf9 cells (ATCC CRL1711), Sf21 cells [the above, J. L. Vaughn et al., In Vitro, Vol. 13, 213-217 (1977)], etc. are used as said Sf cells.

Monkey COS-7 cells, Vero cells, Chinese hamster cells CHO, DHFR gene-deficient Chinese hamster cells CHO (dhfr- CHO cells), mouse L cells, mouse 3T3 cells, mouse myeloma cells, human HEK293 cells, human FL cells, 293 cells, C127 cells, BALB3 T3 cells, Sp-2/O cells, etc., are used as animal cells.

Organisms of the genus Escherichia are transformed, for example, by the methods described in Proc. Natl. Acad. Sci. USA, Vol. 69, 2110 (1972) and Gene, Vol. 17, 107 (1982).

Organisms of the genus Bacillus are transformed, for example, by the methods described in Molecular & General Genetics, Vol. 168, 111 (1979).

Yeasts are transformed, for example, by the methods described in Proc. Natl. Acad. Sci. USA, Vol. 75, 1929 (1978).

[0018]

Insect cells or insects are transformed, for example, by the methods described in Bio/Technology, Vol. 6, pp. 47-55 (1988).

Animal cells are transformed, for example, by the methods described in Virology, Vol. 52, 456 (1973).

Examples of methods of introducing the expression vector into cells include lipofection [P. L. Felgner et al., Proceedings of the National Academy of Sciences of the United States of America, Vol. 84, p. 7413 (1987)], the calcium phosphate method [F. L. Graham and A. J. van der Eb, Virology, Vol. 52, pp. 456-467 (1973)], and electroporation [E. Neumann et al., EMBO Journal, Vol. 1, pp. 841-845 (1982)].

Transformants transformed by the expression vector that contains the DNA encoding CD72 or CD100 used in the present invention are obtained in this way.

A method of stably expressing the CD72 or CD100, etc. used in the present invention using animal cells is selection by clone selection of cells that have incorporated the expression vector that was introduced into the aforementioned animal cells into the chromosomes. Specifically, transformants are selected using the

aforementioned selection markers as the indicator. Furthermore, stable animal cell lines that have high expression capacity for the polypeptide, etc. can be obtained by repeated clone selection of the animal cells obtained using selection markers in this way. When the dhfr gene is used as the selection marker, animal cell lines with even higher expression can be obtained by intracellular amplification of the DNA encoding the polypeptide or partial peptides thereof together with the dhfr gene by gradually raising the MTX concentration during culture and selecting resistant lines.

The polypeptide, etc. can be manufactured by producing and accumulating the polypeptide, etc. by culturing the aforementioned transformants under conditions that permit expression of the DNA encoding the polypeptide, etc. (CD72, CD100).

A liquid medium is appropriate as the medium used in culture when culturing transformants in which the host is an organism of the genera Escherichia or Bacillus. The medium contains a carbon source, nitrogen source, inorganic material, etc., necessary for the growth of said transformants. Examples of carbon sources include glucose, dextrin, soluble starch, and sucrose. Examples of nitrogen sources include organic and inorganic materials such as ammonium salts, nitrate, corn steep liquor, peptone, casein, meat extract, soybean meal, and potato extract. Examples of inorganic materials include calcium chloride, sodium dihydrogen phosphate, and magnesium chloride. Yeast, vitamins, growth accelerators, etc. may also be added. The pH of the medium is preferably approximately 5-8.

[0019]

M9 medium that contains glucose and casamino acid [Miller, Journal of Experiments in Molecular Genetics, 431-433, Cold Spring Harbor Laboratory, New York, 1972], for example, is preferred as the medium when culturing organisms of the genus Escherichia. Drugs such as 3β, indolylacrylic acid, for example, can be added if necessary to make the promoter act more efficiently.

When the host is an organism of the genus Escherichia, culture is usually conducted for approximately 3-24 hours at approximately 15-43°C. Ventilation or agitation can also be added as necessary.

When the host is an organism of the genus Bacillus, culture is usually conducted for approximately 6-24 hours at approximately 30-40°C. Ventilation or agitation can also be added as necessary.

When culturing transformants in which the host is a yeast, examples of the medium include Burkholder minimal medium [K. L. Bostian et al., Proc. Natl. Acad. Sci. USA, Vol. 77, 4505 (1980)] and SD medium that contains 0.5% casamino acid [G. A. Bitter et al., Proc. Natl. Acad. Sci. USA, Vol. 81, 5330 (1984)]. The pH of the medium is preferably adjusted to approximately 5-8. Culture is usually performed for approximately 24-72 hours at approximately 20-35°C, with aeration or agitation added as necessary.

[0020]

When culturing transformants in which the host is an insect cell, medium obtained by appropriate addition of additives such as immobilized 10% bovine serum, etc. to Grace's insect medium (T.C.C. Grace, Nature 195, 788 (1962)) is used as the medium. The pH of the medium is preferably adjusted to approximately 6.2-6.4. Culture is usually performed for approximately 3-5 days at approximately 27°C, with aeration or agitation added as necessary.

When culturing transformants in which the host is an animal cell, MEM medium containing approximately 5-20% fetal calf serum [Science, Vol. 122, 501 (1952)], DMEM medium [Virology, Vol. 8, 396 (1959)], RPMI 1640 medium [The Journal of the American Medical Association, Vol. 199, 519 (1967)], 199 medium [Proceeding of The Society for the Biological Medicine [sic], Vol. 73, 1 (1950)], etc., are used as the medium. The pH is preferably approximately 6-8. Culture is usually performed for approximately 15-60 hours at approximately 30-40°C, with aeration or agitation added as necessary.

When CHO (dhfr) cells and the dhfr gene as a selection marker are used in particular, it is preferable to use basically thymidine-free DMEM medium that contains dialyzed fetal calf serum.

[0021]

The CD72 and CD100 used in the present invention can be isolated and purified from the aforementioned culture, for example, by the following methods.

When extracting the CD72 or CD100 used in the present invention from the cultured mass or cells, the mass or cells are collected by a known method after culture, suspended in a suitable buffer, and disrupted by a means such as ultrasonication, lysozyme and/or freezing-thawing. A means of obtaining a crude extract of polypeptide can then be employed, such as centrifugation or filtration. The buffer may contain protein denaturing agents such as urea or guanidine hydrochloride or surfactants such as Triton X-100 (registered trademark, sometimes abbreviated hereinafter as TM).

When the CD72 or CD100 used in the present invention is secreted in the culture broth, the cell mass or cells are separated from the supernatant by a known method after culture has been completed and the supernatant collected.

The CD72 or CD100 used in the present invention contained in the culture supernatant or extract obtained in this way can be purified by an appropriate combination of known isolation and purification techniques. Techniques that utilize solubility such as salting out and solvent precipitation, techniques that utilize primarily differences in molecular weight such as dialysis, ultrafiltration, gel filtration, and SDS-polyacrylamide gel electrophoresis, techniques that utilize differences in charge such as ion-exchange chromatography, techniques that utilize specific affinity such as affinity chromatography, techniques that utilize differences in hydrophobicity such as reverse-phase high-performance liquid chromatography, and techniques that utilize differences in isoelectric point such as

isoelectrophoresis and chromatofocusing are used as these known isolation and purification techniques.

[0022]

When the CD72 or CD100 used in the present invention obtained in this way has been obtained as a free compound, it can be converted into a salt by or in accordance with known methods. Conversely, when it has been obtained in the form of a salt, it can be converted into a free compound or another salt by or in accordance with known methods.

The polypeptide can also be partially removed by applying arbitrary modifications by causing appropriate protein-modifying enzymes to act before or after purification on the CD72 or CD100 used in the present invention to produce recombinants. For example, trypsin, chymotrypsin, arginyl endopeptidase, protein kinase, glycosidase, etc., are used as protein-modifying enzymes.

The presence of the polypeptide of the present invention produced in this way can be measured by enzyme immunoassay using specific antibodies, etc.

[0023]

A screening method for compounds or salts thereof that change the binding property between CD100 or salts thereof and CD72 or salts thereof characterized by using CD100 or salts thereof and CD72 or salts thereof and a screening kit for compounds or salts thereof that change the binding property between CD100 or salts thereof and CD72 or salts thereof characterized by using CD100 or salts thereof and CD72 or salts thereof (abbreviated hereinafter as screening method of the present invention and screening kit of the present invention) will be described in detail below.

Compounds (e.g., peptides, proteins, nonpeptidic compounds, synthetic compounds, fermentation products, etc.) or salts thereof that change the binding property between CD100 or salts thereof and CD72 or salts thereof can be screened by using CD72 or salt thereof as the receptor or by constructing a recombinant CD72 expression system and using a binding assay system with CD100 or

salts thereof (ligand-receptor assay system) that employs said expression system.

Such compounds include compounds that have CD72-mediated antibody production promoting activity [e.g., promoting or inhibiting activity for antibody production, intracellular Ca^{2+} release, intracellular cAMP production, production of inositol phosphate, changes of cell membrane potential, phosphorylation of intracellular proteins, c-fos activation, pH reduction and others] (i.e., CD72 agonists) and compounds that do not have said antibody production promoting activity (i.e., CD72 antagonists). The term "changes the binding property between CD100 or salts thereof and receptors thereof (e.g., CD72 or salts thereof)" encompasses both inhibition of binding between CD100 or salts thereof and receptors thereof (e.g., CD72 or salts thereof) and promotion of binding with the ligand.

[0024]

Specifically, the present invention proposes a screening method for compounds or salts thereof that change the binding property between CD100 or salts thereof and CD72 or salts thereof characterized by comparing (i) when CD100 or salt thereof is brought into contact with CD72 or salt thereof and (ii) when CD100 or salt thereof and the test compound are brought into contact with the aforementioned CD72 or salt thereof.

Comparison of (i) when CD100 or salt thereof is brought into contact with CD72 or salt thereof as above and (ii) when CD100 or salt thereof and the test compound are brought into contact with CD72 or salt thereof as above is done, for example, by measuring the antibody production promoting activity and the level of ligand binding to said CD72 or salt thereof in the screening method of the present invention.

[0025]

Specifically, the screening method of the present invention is:

(1) A screening method for compounds or salts thereof that change the binding property between CD100 or

salts thereof and CD72 or salts thereof characterized by measuring and comparing the level of binding of labeled CD100 or salt thereof to said CD72 or salt thereof when labeled CD100 or salt thereof is brought into contact with CD72 or salt thereof as above and when labeled CD100 or salt thereof and the test compound are brought into contact with CD72 or salt thereof,

(2) A screening method for compounds or salts thereof that change the binding property between CD100 or salts thereof and CD72 or salts thereof characterized by measuring and comparing the level of binding of labeled CD100 or salt thereof to said cells or said membrane fraction when labeled CD100 or salt thereof is brought into contact with cells or a membrane fraction of said cells that contains CD72 or salt thereof and when labeled CD100 or salt thereof and the test compound are brought into contact with cells or a membrane fraction of said cells that contain CD72 or salt thereof,

(3) A screening method for compounds or salts thereof that change the binding property between CD100 or salts thereof and CD72 or salts thereof characterized by measuring and comparing the level of binding of labeled CD100 or salt thereof to CD72 or salt thereof when labeled CD100 or salt thereof is brought into contact with CD72 or salt thereof expressed on the cell membrane by culturing transformants that contain DNA encoding CD72 and when labeled CD100 or salt thereof and the test compound are brought into contact with CD72 or salt thereof expressed on the cell membrane by culturing transformants that contain DNA encoding CD72,

[0026]

(4) A screening method for compounds or salts thereof that change the binding property between CD100 or salts thereof and CD72 or salts thereof characterized by measuring and comparing the antibody production promoting activity mediated by CD72 or salt thereof [e.g., promoting or inhibiting activity for release of arachidonic acid, release of acethylcholine, intracellular Ca^{2+} release, intracellular cAMP production,

intracellular cGMP production, production of inositol phosphate, changes of cell membrane potential, phosphorylation of intracellular proteins, c-fos activation, pH reduction and others] when a compound that activates CD72 or salts thereof (e.g., CD100 or salts thereof) is brought into contact with cells that contain CD72 or salt thereof and when a compound that activates CD72 or salts thereof and the test compound are brought into contact with cells that contain CD72 or salt thereof, and

(5) A screening method for compounds or salts thereof that change the binding property between CD100 or salts thereof and CD72 or salts thereof characterized by measuring and comparing the antibody production promoting activity mediated by CD72 or salts thereof [e.g., promoting or inhibiting activity for antibody production, intracellular Ca^{2+} release, intracellular cAMP production, production of inositol phosphate, changes of cell membrane potential, phosphorylation of intracellular proteins, c-fos activation, pH reduction and others] when a compound that activates CD72 or salts thereof (e.g., CD100 or salt thereof, etc.) is brought into contact with CD72 or salt thereof expressed on the cell membrane by culturing transformants that contain DNA encoding CD72 and when a compound that activates CD72 or salts thereof and the test compound are brought into contact with CD72 or salt thereof expressed on the cell membrane by culturing transformants that contain DNA encoding CD72.

[0027]

The screening method of the present invention will be explained specifically below.

First, any may be used as the CD72 employed in the screening method of the present invention as long as it contains the aforementioned CD72. Membrane fractions of organs of humans, warm-blooded animals, fish, etc., are appropriate. However, since it is extremely difficult to obtain organs from humans, it is appropriate to use CD72 or salts thereof expressed in large quantities using recombinants as that used in screening.

The method described above and the like are used in the production of CD72 and salts thereof.

When cells or membrane fractions of said cells that contain CD72 or salts thereof are used in the screening method of the present invention, they may be prepared as described below.

When cells that contain CD72 or salts thereof are used, said cells may be immobilized by glutaraldehyde, formalin, etc. Immobilization can be performed by a known technique.

Cells that contain CD72 or salts thereof means host cells expressing CD72 or salts thereof. Examples of said host cells include the aforementioned *E. coli*, *B. subtilis*, yeasts, insect cells, and animal cells.

The membrane fraction means the fraction that contains a large amount of cell membrane obtained by a known method after disrupting the cells. Examples of the means of disrupting the cells include crushing by Potter-Elvehjem homogenizer, disrupting by Waring Blender or polytron (made by Kinematica), disrupting by ultrasonication, and disrupting by spraying the cells from a fine nozzle while pressing by French press, etc. Primarily fractionation by centrifugation such as fractionation centrifugation and density gradient centrifugation are used in fractionation of the cell membrane. For example, the disrupted cell solution is centrifuged for a short time (usually approximately 1-10 minutes) at low speed (500-3000 rpm). The supernatant is then centrifuged for 30 minutes to 2 hours at high speed (15,000-30,000 rpm) and the precipitate obtained taken as the membrane fraction. Said membrane fraction contains large amounts of the expressed CD72 or salt thereof and cell-derived membrane components such as phospholipids and membrane proteins.

[0028]

The amount of CD72 or salt thereof in said cells or membrane fraction that contains CD72 or salts thereof is preferably 10^3-10^8 molecules per cell, appropriately 10^5-10^7 molecules appropriately. A higher level of

expression not only raises the ligand binding activity relative to the membrane fraction (relative activity) and makes it possible to construct a high-sensitivity screening system, but allows measurement of a greater number of samples in the same lot.

An appropriate CD72 fraction and labeled ligand (CD100) are used to implement the aforementioned methods (1)-(3) to screen compounds that change the binding property between CD100 or salts thereof and CD72 or salts thereof. The CD72 fraction is preferably a natural CD72 fraction or a recombinant CD72 fraction with equivalent activity or the like. Here, the term equivalent activity means equivalent ligand binding activity, etc. A labeled ligand (CD100) or labeled ligand (CD100) analog compound, etc. is used as the labeled ligand. For example, labeled ligands (CD100) labeled by [^{3}H], [^{125}I], [^{14}C], [^{35}S], etc., can be utilized. In particular, labeled CD100 or CD100 derivatives prepared by publicly known methods using Bolton-Hunter reagent can also be utilized.

Specifically, when screening compounds that change the binding property between CD100 or salts thereof and CD72 or salts thereof, a receptor standard is prepared by suspending cells or a membrane fraction of cells that contain CD72 or salt thereof in a buffer appropriate for screening. The buffer may be any that does not inhibit the binding of the ligand and receptor such as pH 4-10 (preferably 6-8) phosphate buffer or Tris-hydrochloride buffer. Surfactants such as CHAPS, Tween-80™ (Kao-Atlas), digitonin, deoxycholate, etc., can also be added to the buffer to reduce nonspecific binding. Furthermore, protease inhibitors such as PMSF, leupeptin, E-64 (made by Peptide Institute), and pepstatin can also be added to suppress protease-induced degradation of CD72 and CD100. A set amount (5000-500,000 cpm) of labeled CD100 is added to 0.01-10 mL of said receptor solution. 10^{-4} - 10^{1} μM of the test compound is made to be present simultaneously. A test tube with an excess of unlabeled CD100 or salt thereof is also used to investigate nonspecific binding (NSB). The reaction is performed for from 20 minutes to

24 hours, preferably 30 minutes to 3 hours, at from 0 to 50°C, preferably 4 to 37°C. After the reaction, the solution is filtered by glass fiber filter paper or the like and washed with an appropriate amount of the same buffer. The radioactivity remaining in the glass fiber filter paper is measured by liquid scintillation counter or γ-counter. Test compounds with specific binding (B-NSB) of, for example, no more than 50% can be selected as candidate compounds with antagonistic inhibition potential when the count (B_0-NSB) obtained by subtracting the nonspecific binding (NSB) from the count with no antagonist present (B_0) is taken as 100%.

BIAcore (made by Amersham Pharmacia Biotech) can also be used to measure the binding of CD72 or salt thereof and CD100 or salt thereof. In this method, CD100 or salt thereof or derivative thereof is immobilized on the sensor chip by amino coupling according to the instructions included with the equipment. A buffer such as phosphate buffer or Tris buffer that contains CD72 or salt thereof or membrane fraction that contains CD72 or salt thereof purified from cells that contain CD72 or salt thereof or transformants that contain DNA encoding CD72 or that contains purified CD72 or salt thereof or membrane fraction that contains CD72 or salt thereof and test compound is passed over the sensor chip at a flow rate of 2-20 μL/min. Compounds that change the binding property between CD72 or salts thereof and CD100 or salts thereof can be screened by observing how the presence of the test compound affects the changes in surface plasmon resonance produced by binding of CD100 or salt thereof and CD72 or salt thereof on the sensor chip. This method is also capable of similar measurement when CD72 or salt thereof is immobilized on the sensor chip and a buffer such as phosphate buffer or Tris buffer that contains CD100 or salt thereof or CD100 or salt thereof and test compound is passed over the sensor chip. Examples of the test compounds are the same as above.

[0029]

To implement the aforementioned methods (4) and (5) to screen compounds that change the binding property between CD100 or salts thereof and CD72 or salts thereof, the antibody production promoting activity mediated by CD72 or salt thereof [e.g., promoting or inhibiting activity for release of arachidonic acid, release of acethylcholine, intracellular Ca^{2+} release, intracellular cAMP production, intracellular cGMP production, production of inositol phosphate, changes of cell membrane potential, phosphorylation of intracellular proteins, c-fos activation, pH reduction and others] can be measured by publicly known methods or using commercial measurement kits. Specifically, cells that contain CD72 or salt thereof are first cultured in multiwell plates or the like. Prior to conducting screening, the medium is exchanged for fresh medium or a suitable buffer that is nontoxic to the cells. After adding the test compound and incubating for a set length of time, the cells are extracted or the supernatant recovered and the products generated assayed by the respective methods. When degrading enzymes present in the cells make it difficult to study production of compounds that employ antibody production promoting activity as the indicator (e.g., arachidonic acid, etc.), assay may be performed after adding inhibitors to said degrading enzymes. As to cAMP production inhibiting activity, it can be detected as a production inhibiting reaction against cells that base line of the production is potentiated using forskoline.

Appropriate cells expressing CD72 or salt thereof are used in screening by measuring of the antibody production promoting activity. The aforementioned recombinant CD72-expressing cell lines are preferred as cells expressing CD72 or salts thereof. It does not matter whether the CD72-expressing cells that are transformants are a stable expression line or a transient expression line. The types of animal cells used are also the same as above.

Examples of test compounds include peptides, proteins, nonpeptide compounds, synthetic compounds,

fermentation products, cell extracts, plant extracts, animal tissue extracts, etc.

[0030]

The following assay system will be used to more specifically explain the above ligand-receptor assay system.

(1) Stimulation of receptor-expressing cells by a receptor agonist causes intracellular class switch and accelerates the production and secretion of all classes of antibodies (IgG, IgA, IgD, IgE) other than IgM. The antibody production-promoting activity of the receptor agonist can be measured directly or indirectly using labeled Ig antibodies by ELISA measurement of the antibody levels produced and secreted. The antibody production-promoting effect of CD100 on cells expressing CD72 can be measured by utilizing this reaction. Specifically, this is done by or in accordance with the method of example 2 discussed below. There, compounds that change the binding property between CD100 or salts thereof and CD72 or salts thereof can be screened by observing changes in antibody production-promoting activity when CD100 or salt thereof or CD100 or salt thereof and test compound are added in comparison to when CD100 or salt thereof is administered alone. Compounds that suppress the antibody production-promoting effect of CD100 on cells expressing CD72 can be selected as candidates with antagonist inhibition potential. On the other hand, agonists can also be screened by administering the test compound alone and observing the antibody production-promoting effect on the cells expressing CD72.

An example of the screening method will be discussed specifically below. 1×10^5 cells/well of spleen resting B cells, prepared by the method described below in example 2, are immobilized by p-formaldehyde together with anti-CD40 monoclonal antibody and 100 units/mL of IL-4 and cultured for approximately 7 days in flat-bottomed 96-well microtiter plates in the presence of normal CHO cells expressing CD100 (2×10^4 cells/well). The IgM or

IgG1 immunoglobulin production is measured by ELISA. Specifically, the culture broth or the control IgM or IgG diluted using 0.1M carbonate buffer (pH 9.6) is injected in 100 μL aliquots into each well of an EIA 96-well immunoplate (MaxiSorp: Nunc) and adhered by standing overnight at approximately 4°C. After washing each well with buffer A (0.02M phosphate buffer, pH 7.0, containing 0.15M NaCl), 100 μL of enzyme-labeled anti-IgM, IgG, IgA, IgD, IgE antibody solution diluted by buffer B (0.02M phosphate buffer, pH 7.0, containing 0.1% BSA and 0.15M NaCl) is added and reacted for another approximately 2 hours at 25°C. Each well is then washed with buffer A, 100 μL of alkali phosphatase substrate solution (1 mg/mL phosphatase substrate (Sigma), 100 mM Tris (pH 9.5), 100 mM NaCl, 5 mM $MgCl_2$) added and reacted for 30 minutes at 25°C. The 405 nm absorbance is measured using an automatic microplate colorimeter. Taking the absorbance of the experiment with only CD100 or salt thereof added as 100% and the absorbance of the experiment with no CD100 or salt thereof added as 0%, the effect of the test compound on the promotion of antibody production by CD100 or salt thereof is calculated. Test compounds with antibody production-promoting activity, for example, of no more than 50% can be selected as candidates with antagonistic inhibition potential.

[0031]

A screening kit for compounds or salts thereof that change the binding property between CD100 or salt thereof and CD72 or salt thereof is one that contains CD72 or salt thereof, cells that contain CD72 or salt thereof or membrane fraction of cells that contain CD72 or salt thereof, and CD100 or salt thereof.

The following can be given as examples of screening kits of the present invention.

1. Screening reagents

(1) Buffer for measurement and buffer for washing

Hanks' Balanced Salt Solution (made by Gibco) with 0.05% bovine serum albumin (made by Sigma) added.

This may be filtered sterilized by a filter with a pore diameter of 0.45 μm and stored at 4°C or may be prepared at the time of use.

(2) CD72 standard

CHO cells expressing CD72 or salt thereof subcultured at 5×10^5 cells/well in 12-well plates and cultured for 2 days at 37°C in 5% CO_2 and 95% air.

(3) Labeled ligand

CD100 or salt thereof labeled by [^{3}H], [^{125}I], [^{14}C], [^{35}S], etc.

This is dissolved in a suitable solvent or buffer, stored at 4°C or -20°C, and diluted to 1 μM by buffer for measurement at the time of use.

(4) Ligand labeling solution

CD100 or salt thereof is dissolved to make 1 mM in PBS that contains 0.1% bovine serum albumin (made by Sigma) and stored at -20°C.

[0032]

2. Measurement method

(1) After washing cells that have been made to express CD72 or salt thereof cultured in 12-well tissue culture plates twice by 1 mL of buffer for measurement, 490 μL of buffer for measurement is added to each well.

(2) After adding 5 μL of 10^{-3}-10^{-10} M test compound solution, 5 μL of labeled CD100 or salt thereof is added and reacted for 1 hour at room temperature. 5 μL of 10^{-3} M ligand is added instead of test compound to investigate the nonspecific binding.

(3) The reaction solution is removed. The plates are washed three times by 1 mL of buffer for washing. The labeled ligand bonded to the cells is dissolved by 0.2N NaOH-1% SDS and mixed with 4 mL of liquid scintillator A (made by Wako Junyaku).

(4) The radioactivity is measured using a liquid scintillation counter (made by Beckman) and the percent maximum binding (PMB) determined by the following formula.

[Formula 1]

$$PMB = (B - NSB) / (B_0 - NSB) \times 100$$

PMB: percent maximum binding

B: a value with specimen added

NSB: nonspecific binding

B_0: maximum binding

[0033]

The compounds or salts thereof obtained using the screening method or screening kit of the present invention are compounds that change the binding property between CD100 or salts thereof and CD72 or salts thereof (inhibit or promote binding). Specifically, they are compounds or salts that have an antibody production promoting activity mediated by CD72 or salt thereof (so-called CD72 agonists) or compounds that do not have said antibody production promoting activity (so-called CD72 antagonists). Examples of said compounds include peptides, proteins, nonpeptide compounds, synthetic compounds, and fermentation products. These compounds may be novel compounds or known compounds.

Concrete methods of evaluating whether a compound is a CD72 agonist or antagonist as above include (i) and (ii) below.

(i) After performing binding assay by a screening method of (1)-(3) above and obtaining a compound that changes the binding property between CD100 or salt thereof and CD72 or salt thereof (particularly one that inhibits binding), one measures whether or not said compound has the aforementioned CD72-mediated antibody production promoting activity. Compounds or salts thereof that have antibody production promoting activity are CD72 agonists and compounds or salts thereof that do not have said activity are CD72 antagonists.

(ii) (a) The test compound is brought into contact with cells that contain CD72 or salt thereof and the aforementioned antibody production promoting activity mediated by CD72 or salt thereof is measured. Compounds or salts thereof with antibody production promoting activity are CD72 agonists.

(b) The antibody production promoting activity mediated by CD72 or salt thereof is measured and compared

when a compound that activates CD72 or salt thereof (e.g., CD100 or CD72 agonist, etc.) is brought into contact with cells that contain CD72 or salt thereof and when a compound that activates CD72 or salt thereof and test compound are brought into contact with cells that contain CD72 or salt thereof. Compounds or salts thereof capable of decreasing the antibody production promoting activity of compounds that activate CD72 or salts thereof are CD72 antagonists.

Said CD72 agonists are useful as safe, low-toxicity drugs in the same way as CD100 or salts thereof because they have similar physiological activity to CD100 and salts thereof on CD72 and salts thereof.

Conversely, CD72 antagonists are useful as safe, low-toxicity drugs that suppress said receptor activity because they are capable of suppressing the physiological activity of CD100 and salts thereof on CD72 and salts thereof.

Moreover, CD72 and salts thereof are useful as safe, low-toxicity drugs because they are capable of suppressing the physiological activity of CD100 and salts thereof in the same way as CD72 antagonists.

CD100 and salts thereof can be used as antibody production inducers, immunopotentiators and the like because they induce class switch and promote antibody production. Therefore, CD72 agonists, among the compounds obtained using the aforementioned screening method or screening kit, can be used in the prevention and treatment of viral infections and diseases (such as colds, influenza, AIDS, hepatitis, herpes, measles, varicella, foot-and-mouth disease, herpes zoster, erythema infectiosum, rubella, exanthema subitum, viral conjunctivitis, viral meningitis, viral pneumonia, viral encephalitis, Lassa fever, Ebola hemorrhagic fever, Marburg disease, Congo hemorrhagic fever, yellow fever, dengue fever, rabies, adult T cell leukemia (ATL), rotavirus infection, polio, mumps, etc.), infections and diseases caused by bacteria or fungi (such as bacterial food poisoning, bacterial diarrhea, tuberculosis,

Hansen's disease, dysentery, typhoid fever, cholera, paratyphus, plague, tetanus, tularemia, brucellosis, anthrax, septicemia, bacterial pneumonia, dermatomycosis, etc.), and cancer (oral cavity cancer, pharyngeal cancer, lip cancer, tongue cancer, gingival cancer, nasopharyngeal cancer, esophageal cancer, stomach cancer, small intestinal cancer, large intestinal cancer including colon cancer, liver cancer, gallbladder cancer, pancreatic cancer, nasal cavity cancer, lung cancer, bone cancer, soft tissue cancer, skin cancer, melanoma, breast cancer, uterine cancer, ovarian cancer, prostate cancer, testicular cancer, cancer of the penis, urinary bladder cancer, kidney cancer, brain tumor, thyroid cancer, lymphoma, leukemia, etc.), etc. CD72 antagonists (or CD72 or salts thereof) can be used in the prevention and treatment of diseases caused by abnormal antibody production or excessive antibody production (such as atopic asthma, allergic rhinitis, atopic dermatitis, allergic bronchitis, pulmonary aspergillosis, parasitic disease, Kimura's disease, hyper-IgE syndrome, Wiskott-Aldrich syndrome, thymic aplasia, Hodgkin's disease, liver cirrhosis, acute hepatitis, chronic rheumatoid arthritis, insulin-dependent diabetes, systemic erythematosus, scleroderma, infertility, endometriosis, autoimmune thyroid disease myasthenia gravis [sic], Hashimoto's disease, Basedow's disease, pernicious anemia, Addison's disease, male infertility, multiple sclerosis, Goodpasture syndrome, pemphigus, pemphigoid, myasthenia gravis, ophthalmia of the lens, sympathetic ophthalmia, autoimmune hemolytic anemia, idiopathic thrombocytopenia, autoimmune leukopenia, Felty syndrome, autoimmune lymphocytopenia, ulcerative colitis, Sjogren's syndrome, systemic autoimmune disease, primary biliary liver cirrhosis, lupoid hepatitis, etc.).

[0034]

Pharmacologically acceptable salts, for example, are used as the salts of compounds obtained using the aforementioned screening method or screening kit. Examples include salts of inorganic bases, salts of

organic bases, salts of inorganic acids, salts of organic acids, and salts of basic and acidic amino acids.

Examples of salts of inorganic bases include alkali metal salts such as sodium and potassium salts, alkaline earth metal salts such as calcium and magnesium salts, aluminum salts, and ammonium salts.

Examples of suitable salts of organic bases include salts of triethylamine, trimethylamine, pyridine, picoline, 2,6-lutidine, ethanolamine, diethanolamine, triethanolamine, cyclohexylamine, dicyclohexylamine, and N,N'-dibenzylethylenediamine.

Examples of suitable salts of inorganic acids include salts of hydrochloric acid, hydrobromic acid, sulfuric acid, and phosphoric acid.

Examples of suitable salts of organic acids include salts of formic acid, acetic acid, propionic acid, fumaric acid, oxalic acid, tartaric acid, maleic acid, citric acid, succinic acid, malic acid, methanesulfonic acid, benzenesulfonic acid, and benzoic acid.

Examples of suitable salts of basic amino acids include salts of arginine, lysine, and ornithine. Examples of suitable salts of acidic amino acids includes salts of aspartic acid and glutamic acid.

Ordinary means can be used when compounds or salts thereof obtained using the screening method or screening kit of the present invention are used as drugs. For example, they can be used orally as tablets, sugar- or enteric-coated if necessary, capsules, elixirs, microcapsules, etc., or nonorally as injections such as suspensions or sterile solutions of water or other pharmacologically acceptable solutions. For example, drugs can be manufactured by mixing said compounds or salts thereof together with physiological carriers, flavorings, excipients, vehicles, preservatives, stabilizers, binders, etc., in common unit-dose form required for drug manufacture. The amount of active ingredient in these preparations is such that an appropriate volume is obtained within the designated range.

[0035]

Binders such as gelatin, corn starch, tragacanth gum, and gum arabic, excipients such as crystalline cellulose, swelling agents such as corn starch, gelatin and alginic acid, lubricants such as magnesium stearate, sweeteners such as sucrose, lactose, and saccharine, and flavorings such as peppermint, wintergreen oil, and cherry, etc., are used as additives that can be mixed with tablets and capsules, etc. When the unit form of the preparation is a capsule, it can also contain a liquid carrier such as an oil in addition to the aforementioned types of materials. Sterile compositions for injection can be formulated in accordance with ordinary drug preparation by dissolution or suspension of the active substance and a naturally-produced vegetable oil such as sesame oil or coconut oil in the vehicle such as water for injection.

[0036]

Examples of aqueous solutions for injection include physiological saline and isotonic solutions that contain glucose and other auxiliaries (e.g., D-sorbitol, D-mannitol, sodium chloride, etc.). Alcohols (e.g., ethanol), polyalcohols (e.g., propylene glycol, polyethylene glycol), nonionic surfactants (e.g., Polysorbate 80™, HCO-50), etc. may be used in combination as suitable dissolution auxiliaries. Examples of oily solutions include sesame oil and soybean oil. Benzyl benzoate, benzyl alcohol, etc. may be used in combination as dissolution auxiliaries.

[0037]

Buffers (e.g., phosphate buffer, sodium acetate buffer), analgesics (e.g., benzalkonium chloride, procaine hydrochloride, etc.), stabilizers (e.g., human serum albumin, polyethylene glycol, etc.), preservatives (e.g., benzyl alcohol, phenol, etc.), antioxidants, etc., may also be combined. The prepared injectable solution is usually packaged in appropriate ampules.

[0038]

The preparations obtained in this way can be administered, for example, to humans and mammals (e.g.,

mice, rats, guinea pigs, rabbits, sheep, pigs, cows, cats, dogs, monkeys, chimpanzees, etc.) because they are safe and low in toxicity.

The dose of the compounds or salts thereof obtained using the screening method or screening kit of the present invention varies depending on the symptoms, etc. However, in oral administration to adults with obesity (body weight 60 kg), it is generally from approximately 0.1 to 1000 mg, preferably approximately 1.0 to 300 mg, more preferably approximately 3.0 to 50 mg, per day. In non-oral administration, the one-time dose also differs depending on the reason for administration, subject organ, symptoms, administration method, etc. However, for example, it is usually appropriate in administration to an adult (body weight 60 kg) in the form of an injection to administer from approximately 0.01 to 30 mg, preferably approximately 0.1 to 20 mg, more preferably approximately 0.1 to 10 mg, per day by intravenous injection. Other animals can also be administered doses calculated per 60 kg.

When CD72 or salts thereof are used as drugs, they can be made into preparations and used in the same way as when compounds or salts thereof obtained by the screening method or screening kit of the present invention as described above are used as drugs.

Abbreviations of the bases and amino acids, etc. in this specification and drawings are based on the IUPAC-IUB Commission on Biochemical Nomenclature abbreviations and abbreviations commonly used in this field. Examples are given below. When amino acids can exist as optical isomers, the L-form is shown unless specifically stated otherwise.

[0039]

DNA:	deoxyribonucleic acid
CDNA:	complementary deoxyribonucleic acid
A:	adenine
T:	thymine
G:	guanine
C:	cytosine

Y: thymine or cytosine

N: thymine, cytosine, adenine, or guanine

R: adenine or guanine

M: cytosine or adenine

W: thymine or adenine

S: cytosine or guanine

RNA: ribonucleic acid

MRNA: messenger ribonucleic acid

DATP: deoxyadenosine triphosphate

DTTP: deoxythymidine triphosphate

DGTP: deoxyguanosine triphosphate

DCTP: deoxycytidine triphosphate

ATP: adenosine triphosphate

EDTA: ethylenediamine tetraacetate

SDS: sodium dodecyl sulfate

EIA: enzyme imunoassay

Gly or G: glycine

Ala or A: alanine

Val or V: valine

Leu or L: leucine

Ile or I: isoleucine

[0040]

Ser or S: serine

Thr or T: threonine

Cys or C: cysteine

Met or M: methionine

Glu or E: glutamic acid

Asp or D: asparatic acid

Lys or K: lysine

Arg or R: arginine

His or H: histidine

Phe or F: phenylalanine

Tyr or Y: tyrosine

Trp or W: tryptophan

Pro or P: proline

Asn or N: asparagine

Gln or Q: glutamine

PGlu: pyroglutamic acid

Me: methyl group

Et: ethyl group

Bu: butyl group

Ph: phenyl group

TC: thiazolydine-4-(R)-carboxamide group

Bom: benzyloxymethyl

NMP: N-methylpyrrolidone

PAM: phenylacetamide methyl

[0041]

The substituents, protective groups, and reagents frequently used in this specification are represented by the following abbreviations

Tos: p-toluenesulfonyl

HONB: N-hydroxy-5-norbornene-2,3-dicarboxyimide

Bzl: benzyl

Cl_2-Bzl: dichlorobenzyl

Z: benzyloxycarbonyl

Br-Z: 2-bromobenzyloxycarbonyl

Cl-Z: 2-chlorobenzyloxycarbonyl

Boc: t-butyloxycarbonyl

HOBT: 1-hydroxybenzotriazole

DCC: N,N'-dicyclohexylcarbodiimide

TFA: trifluoroacetic acid

Fmoc: N-9-fluorenylmethoxycarbonyl

DNP: dinitrophenyl

Bum: tert-butoxymethyl

Tri: trityl

BSA: bovine serum albumin

CHAPS: 3-[(3-cholamido)dimethylammonio]-1-propane sulfonate

E64: (L-3-trans-carboxyoxirane-2-carbonyl)L-leucyl-agmatin

DNP-OVA: dinitrophenyl ovalbumin

DNP-BSA: dinitrophenyl bovine serum albumin

ELISA: enzyme-linked immunosorbent assay

EIA: enzyme immunosorbent assay

PBS: phosphate buffered saline

LPS: lipopolysaccharide

conA: concavalin A

[0042]

The sequence numbers in this specification indicate the following sequences.

[SEQ ID NO: 1] shows the amino acid sequence of mouse CD100.

[SEQ ID NO: 2] shows the base sequence of mouse CD100.

[SEQ ID NO: 3] shows the amino acid sequence of human CD100.

[SEQ ID NO: 4] shows the base sequence of human CD100.

[SEQ ID NO: 5] shows the amino acid sequence of mouse CD72.

[SEQ ID NO: 6] shows the base sequence of mouse CD72.

[SEQ ID NO: 7] shows the amino acid sequence of human CD72.

[SEQ ID NO: 8] shows the base sequence of human CD72.

[SEQ ID NO: 9] shows the base sequence of the primer on the N terminal used to prepare for the mCD100-Fc described in reference example 1.

[SEQ ID NO: 10] shows the base sequence of the primer on the C terminal used to prepare for the mCD100-Fc described in reference example 1.

[0043]

[Examples]

The present invention is explained in more detail below through reference examples and examples. However, these do not limit the scope of the present invention.

[0044]

Reference example 1: Isolation of CD100 with expression enhanced by CD40 stimulation

1×10^8 mouse B cell line WEHI231 cells were stimulated for 8 hours using anti-CD40 antibody amd HM40-3 (PharMingen). The total RNA was isolated by the guanidine isothiocyanate phenol method from unstimulated or stimulated cells and mRNA was purified using oligo(dT)-bonded magnetic beads (Promega). cDNA synthesis and subtraction cloning were performed using a PCR-Select

cDNA subtraction kit (Clontech). The cDNA fragment produced by CD40 stimulation was inserted directly into a T/A vector (Invitrogen). The CD100 gene described in the Journal of Biological Chemistry 271, 33376-33381 (1996) was isolated by comparing the base sequences obtained.

The mCD100-Fc described in examples 1 and 3 below is a protein with solubilized human IgG1 Fc fused to mouse CD100. Specifically, secretion-type mouse CD100 cDNA was prepared from mouse CD100 cDNA extracted from WEHI231 cells that had been stimulated by CD40 by PCR using an oligonucleotide that combined a primer containing a sense-wise SalI site (gctgtcgactgtgtgcccgttgctgaaggcct) [SEQ ID NO: 9] and a primer containing an antisense-wise BamHI site (gacggatcctacttactttgctttgcttgcttgagatacaccgtcttctctga) [SEQ ID NO: 10]. A gene that expresses mCD100-Fc protein was made by inserting the SalI-BamHI fragment obtained into the SalI-BamHI fragment DNA fragment of a pEFBos human IgG1 Fc cassette. Transformants were prepared by inserting this gene into a P3U1 plasmacytoma by electroporation (conducted at 0.25 kV, 960 microFD using a Biorad Gene Pulsar). Specifically, 10^7 cells were transformed by pEFBos-mCD100-Fc plasmid DNA that had been cleaved by 50 μg of HindIII and pMC1neo vector that had been cleaved by BamHI. After culturing for 10 days in RPMI medium that contained 10% fetal calf serum and 0.3 mg/mL of G418, the colonies resistant to G418 were isolated and cloned. mCD100-Fc protein was purified from the culture broth by protein A Sepharose (Amersham Pharmacia).

The biotinylated mCD100-Fc described in example 1 below was obtained by bonding biotin to mC100-Fc by a biotinylation kit (Boehringer-Mannheim). The CHO cells expressing CD100 described in example 2 were transformants obtained by inserting the CD100 gene into CHO cells. They express CD100 protein. Specifically, the entire length of the CD100 cDNA was incorporated into a pEFBOS vector and inserted together with a pMC1neo vector into the CHO cells using LipofectaminePlus (Life

Technologies). The G418-resistant cells were selected after 10 days in the presence of 0.3 mg/mL of G418.

[0045]

Reference example 2: Isolation of molecular CD72 bonded with CD100

2B4 cells from C57BL/6 mice were cultured using RPMI1640 medium that contained 10% fetal calf serum. 1 × 10^6 cells/mL of 2B4 cells were then stimulated for 18 hours by 2 μg/mL of conA. The total RNA was isolated from the cells by guanidine isothiocyanate density gradient centrifugation and the mRNA selected from the total RNA using oligo(dT)-bonded magnetic beads (Promega). Double-stranded cDNA that contained oligo(dT) was synthesized using a SuperScriptII cDNA synthesis kit (Life Technologies). A BstXI adapter (Invitrogen) was added to the cDNA, followed by fractionation by 1% agarose gel electrophoresis. The cDNA of at least 1.0 kb was recovered and inserted into a pME18S vector that had been cleaved by BstXI. *E. coli* DH10B cells (Life Technologies) were transformed by electroporation (conducted at 2.5 kV, 25 μFD using a Biorad Gene Pulsar) using this inserted DNA. COS7 cells were transformed using Lipofectamine Plus using a plasmic obtained from the *E. coli* made from 2 × 10^7 independent clones. After 3 days of transformation, the cells were recovered and resuspended in a concentration of 5 × 10^6 cells/mL in PBS that contained 5% fetal calf serum, 2.5 μg/mL Fc block, and 5 μg/mL biotinylated mCD100-Fc and allowed to stand for 1 hour on ice. The cells were then washed with cold PBS and suspended in PBS that contained Dynabeads with M-280 streptoavidin bonded to them. After 30 minutes in suspension, the cells were washed 10 times by cold PBS using a magnetic particle concentrator. The extrachromosomal plasmid DNA was extracted by the Hirt method (Proceeding of the National Academy of Sciences of USA, 84, 3365-3369 (1987)). The plasmid DNA was inserted into *E. coli* DH10B cells by electroporation (conducted at 0.25 kV, 960 microFD using a Biorad Gene Pulsar) and second, third, and fourth transformations carried out by

protoplast fusion. The above extraction by magnetic force was repeated four times. A clear 1.4 kb band was found as a result. Analysis of the base sequence of this 1.4 kb cDNA clone found it to be the total length of mouse CD72 cDNA [SEQ ID NO: 6].

The CHO cells expressing CD72 described in example 1 are transformants obtained by inserting a CD72 gene into CHO cells. They express CD72 protein. Specifically, a pME18S vector that incorporated CD72 was inserted together with a pMC1neo vector into CHO cells by electroporation (conducted at 0.25 kV, 960 microFD using a Biorad Gene Pulsar). The G418-resistant cells were selected after 10 days in the presence of 0.3 mg/mL of G418.

[0046]

Example 1: Binding of CD100 and CD72

mCD100-Fc was biotinylated using a biotinylation kit. 10^6 control CHO cells and CHO transformants expressing CD72 were reacted with biotinylated mCD100-Fc (40 μg/mL) for 1 hour on ice in stain buffer (PBS containing 2% fetal calf serum, 0.02% sodium azide, 2 mM calcium chloride, and 1 mM magnesium chloride) that contained 5 μg/mL of Fc block (PharMingen) for analysis by flow cytometry. After washing with stain buffer, the cells were stained for 20 minutes by FITC-labeled streptoavidin (Becton Dickinson). The cells were then washed with stain buffer and the cells bonded to the FITC-labeled streptoavidin analyzed by flow cytometer.

The results are shown in Figure 1. The graph on the left shows the control CHO cells and that on the right the CHO cells expressing CD72. The dotted line shows when mCD100-Fc was not added and the solid line when mCD100-Fc was added. The abscissa gives the fluorescence intensity per cell and the ordinate gives the relative cell count. The fluorescence intensity did not change even when biotinylated mCD100-Fc was added to the CHO cells on the left.

This shows that CHO cells do not bind to biotinylated mCD100-Fc.

The fluorescence intensity was stronger when biotinylated mCD100-Fc was added (solid line) than when it was not (dotted line) in the CHO cells expressing CD72 on the right. This illustrates that biotinylated mCD100-Fc binds to CD72 on the surface of CHO cells expressing CD72.

[0047]

Example 2: Class switch-enhancing effect of mouse CD100

Resting B cells from the spleen of C57BL/6 mice prepared in a concentration of 1×10^5 cells/well were added together with 100 units/mL of anti-CD40 monoclonal antibody or IL-4 and p-formaldehyde-immobilized CHO cells expressing CD100 (2×10^4 cells/well) to flat-bottomed 96-well microtiter plates and cultured for 7 days. The production of IgM or IgG1 immunoglobulin was measured by ELISA. Specifically, the culture broth or control IgM or IgG was diluted using 0.1M carbonate buffer (pH 9.6). 100 µL was aliquoted into each well of an EIA 96-well immunoplate (MaxiSorp, Nunc) and adhered by standing overnight at approximately 4°C. After washing each well with buffer A (pH 7.0, 0.02M phosphate buffer that contained 0.15M NaCl), 100 µL of enzyme-labeled anti-IgM or IgG1 antibody solution that had been diluted by buffer B (pH7.0, 0.02M phosphate buffer that contained 0.1% BSA and 0.15M NaCl) was added and reacted at 25°C for another approximately 2 hours. Each well was then washed with buffer A and 100 µL of alkaline phosphatase substrate solution (1 mg/mL of phosphatase substrate (Sigma), 100 mM Tris (pH 9.5), 100 mM NaCl, and 5 mM $MgCl_2$) was added and reacted for 30 minutes at 25°C. The 405 nm absorbance was measured using an automatic microplate colorimeter. The amount of antibody in each reaction solution was assayed by reading against a calibrationn curve of the absorbance and antibody content produced separately by adhering known quantities of IgM and IgG1.

The experiment compared the IgM and IgG1 levels (1) when only medium was added in the absence of insufficient CD100-expressing CHO cells, (2) when only medium was added in the presence of CD100-expressing CHO cells, (3)

when anti-CD40 antibody (αCD40) and IL-4 were added in the absence of CD100-expressing CHO cells, and (4) when anti-CD40 antibody (αCD40) and IL-4 were added in the presence of CD100-expressing CHO cells. The results are shown in Figure 2. The abscissa shows the results of (1), (2), (3) and (4) from left to right. The ordinate shows the antibody level (unit: ng/mL) determined from the absorbance. The presence of CD100 (2) had no effect on antibody production of either IgM or IgG1 in comparison to the unadded control (1). Stimulation by anti-CD40 antibody and IL-4 (3) induced antibody production of both IgM and IgG1 in comparison to the unadded control (1). Stimulation by anti-CD40 antibody and IL-4 in the presence of CD100 (4) raised IgG1 production more than (3) despite a slight decrease in IgM production in comparison to when stimulated by anti-CD40 antibody and IL-4. This illustrates induction of what is called class switch, the phenomenon whereby the class of antibodies produced and secreted by B cells switches from IgM to IgG1.

[0048]

Example 3: In vivo antibody production-enhancing effect of CD100

C57BL/6 mice were immunized by intraperitoneal inoculation of 100 μg of dinitrophenyl ovalbumin (DNP-OVA) prepared in aluminum [sic]. Following immunization, the animals were administered 200 μg/day of human IgG1 myeloma protein or mCD100-Fc for 10 days. Serum was collected 6 and 10 days after DNP-OVA administration. The antibody titer of antibody specific to DNP was measured by ELISA using DNP-BSA. Specifically, the immunized mouse serum was diluted using 0.1M carbonate buffer (pH 9.6). 100 μL was aliquoted into each well of an EIA 96-well immunoplate (MaxiSorp, Nunc) that had been coated by DNP-BSA and adhered by standing overnight at 4°C. After washing each well with buffer A (pH 7.0, 0.02M phosphate buffer that contained 0.15M NaCl), 100 μL of alkaline phosphatase-labeled anti-mouse IgM or IgG1 antibody solution that had been diluted by buffer B (pH7.0, 0.02M

phosphate buffer that contained 25% Blockase (Dainippon Seiyaku) and 0.15M NaCl) was added and reacted for another 2 hours at 25°C to bond to the anti-DNP antibodies adhered to the wells. Each well was then washed with buffer A, 100 μL of alkaline phosphatase substrate solution added and reacted for 30 minutes at 25°C. The 405 nm absorbance was measured using an automatic microplate colorimeter. The DNP-specific antibodies were determined.

The results are shown in Figure 3. The graph on the left shows the antibody titer to DNP contained in the serum 6 days after DNP-OVA administration. The graph on the right shows that in the serum 10 days after administration. The white block on the abscissa shows the antibody titer when IgG1 myeloma protein was administered and the black block that when mCD100-Fc was administered. The ordinate anti-DNP gives the antibody titer to DNP. 1/1000 of the antibody level to DNP contained in the serum of control mice 12 days after administration was taken as one unit. When CD100 (mCD100-Fc) was administered, the antibody titer on day 6 was more than three times higher than the antibody titer on day 6 when the control human IgG1 myeloma protein was administered and surpassed the antibody titer on day 10 when the control human IgG1 myeloma protein was administered.

[0049]

[Effects of the Invention]

The screening method for compounds or salts thereof that change the binding property between CD72 or salts thereof and CD100 or salts thereof of the present invention characterized by using CD72 or salts thereof and CD100 or salts thereof is useful as a screening method for CD72 agonists that can be used as drugs for the prevention or treatment of infections or diseases caused by viruses (such as colds, influenza, AIDS, hepatitis, herpes, measles, varicella, foot-and-mouth disease, herpes zoster, erythema infectiosum, rubella, exanthema subitum, viral conjunctivitis, viral meningitis, viral pneumonia, viral encephalitis, Lassa fever, Ebola

hemorrhagic fever, Marburg disease, Congo hemorrhagic fever, yellow fever, dengue fever, rabies, adult T cell leukemia (ATL), rotavirus infection, polio, mumps, etc.), infections and diseases caused by bacteria or fungi (such as bacterial food poisoning, bacterial diarrhea, tuberculosis, Hansen's disease, dysentery, typhoid fever, cholera, paratyphus, plague, tetanus, tularemia, brucellosis, anthrax, septicemia, bacterial pneumonia, dermatomycosis, etc.), and cancer (oral cavity cancer, pharyngeal cancer, lip cancer, tongue cancer, gingival cancer, nasopharyngeal cancer, esophageal cancer, stomach cancer, small intestinal cancer, large intestinal cancer including colon cancer, liver cancer, gallbladder cancer, pancreatic cancer, nasal cavity cancer, lung cancer, bone cancer, soft tissue cancer, skin cancer, melanoma, breast cancer, uterine cancer, ovarian cancer, prostate cancer, testicular cancer, cancer of the penis, urinary bladder cancer, kidney cancer, brain tumor, thyroid cancer, lymphoma, leukemia, etc.) and CD72 antagonists that can be used as drugs for the prevention and treatment of diseases caused by abnormal antibody production or excessive antibody production (such as atopic asthma, allergic rhinitis, atopic dermatitis, allergic bronchitis, pulmonary aspergillosis, parasitic disease, Kimura's disease, hyper-IgE syndrome, Wiskott-Aldrich syndrome, thymic aplasia, Hodgkin's disease, liver cirrhosis, acute hepatitis, chronic rheumatoid arthritis, insulin-dependent diabetes, systemic erythematosus, scleroderma, infertility, endometriosis, autoimmune thyroid disease myasthenia gravis [sic], Hashimoto's disease, Basedow's disease, pernicious anemia, Addison's disease, male infertility, multiple sclerosis, Goodpasture syndrome, pemphigus, pemphigoid, myasthenia gravis, ophthalmia of the lens, sympathetic ophthalmia, autoimmune hemolytic anemia, idiopathic thrombocytopenia, autoimmune leukopenia, Felty syndrome, autoimmune lymphocytopenia, ulcerative colitis, Sjogren's syndrome, systemic autoimmune disease, primary biliary liver cirrhosis, lupoid hepatitis, etc.)

[0050]

[Sequence Listing]

SEQUENCE LISTING

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Asn Tyr Ser Ala Leu Leu Met Ser Glu Asp Lys Asp Thr Leu Tyr Val
50 55 60
Gly Ala Arg Glu Ala Val Phe Ala Val Asn Ala Leu Asn Ile Ser Glu
65 70 75 80
Lys Gln His Glu Val Tyr Trp Lys Val Ser Glu Asp Lys Lys Ser Lys
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Cys Ala Glu Lys Gly Lys Ser Lys Gln Thr Glu Cys Leu Asn Tyr Ile
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Arg Val Leu Gln Pro Leu Ser Ser Thr Ser Leu Tyr Val Cys Gly Thr
115 120 125
Asn Ala Phe Gln Pro Thr Cys Asp His Leu Asn Leu Thr Ser Phe Lys
130 135 140
Phe Leu Gly Lys Ser Glu Asp Gly Lys Gly Arg Cys Pro Phe Asp Pro
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Ala His Ser Tyr Thr Ser Val Met Val Gly Gly Glu Leu Tyr Ser Gly
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Thr Ser Tyr Asn Phe Leu Gly Ser Glu Pro Ile Ile Ser Arg Asn Ser

180 185 190

Ser His Ser Pro Leu Arg Thr Glu Tyr Ala Ile Pro Trp Leu Asn Glu

195 200 205

Pro Ser Phe Val Phe Ala Asp Val Ile Gln Lys Ser Pro Asp Gly Pro

210 215 220

Glu Gly Glu Asp Asp Lys Val Tyr Phe Phe Phe Thr Glu Val Ser Val

225 230 235 240

Glu Tyr Glu Phe Val Phe Lys Leu Met Ile Pro Arg Val Ala Arg Val

245 250 255

Cys Lys Gly Asp Gln Gly Gly Leu Arg Thr Leu Gln Lys Lys Trp Thr

260 265 270

Ser Phe Leu Lys Ala Arg Leu Ile Cys Ser Lys Pro Asp Ser Gly Leu

275 280 285

Val Phe Asn Ile Leu Gln Asp Val Phe Val Leu Arg Ala Pro Gly Leu

290 295 300

Lys Glu Pro Val Phe Tyr Ala Val Phe Thr Pro Gln Leu Asn Asn Val

305 310 315 320

Gly Leu Ser Ala Val Cys Ala Tyr Thr Leu Ala Thr Val Glu Ala Val

325 330 335

Phe Ser Arg Gly Lys Tyr Met Gln Ser Ala Thr Val Glu Gln Ser His

340 345 350

Thr Lys Trp Val Arg Tyr Asn Gly Pro Val Pro Thr Pro Arg Pro Gly

355 360 365

Ala Cys Ile Asp Ser Glu Ala Arg Ala Ala Asn Tyr Thr Ser Ser Leu

370 375 380

Asn Leu Pro Asp Lys Thr Leu Gln Phe Val Lys Asp His Pro Leu Met

385 390 395 400

Asp Asp Ser Val Thr Pro Ile Asp Asn Arg Pro Lys Leu Ile Lys Lys

405 410 415

Asp Val Asn Tyr Thr Gln Ile Val Val Asp Arg Thr Gln Ala Leu Asp

420 425 430

Gly Thr Phe Tyr Asp Val Met Phe Ile Ser Thr Asp Arg Gly Ala Leu

435 440 445

His Lys Ala Val Ile Leu Thr Lys Glu Val His Val Ile Glu Glu Thr

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Gln Leu Phe Arg Asp Phe Glu Pro Val Leu Thr Leu Leu Leu Ser Ser

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Lys Lys Gly Arg Lys Phe Val Tyr Ala Gly Ser Asn Ser Gly Val Val

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705 710 715 720
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755 760 765
Ala Leu Leu Leu Gly Lys Lys Thr Pro Lys Ser Asp Phe Ser Asp Leu
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Glu Gln Ser Val Lys Glu Thr Leu Val Glu Pro Gly Ser Phe Ser Gln
785 790 795 800
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805 810 815

Glu Gln Asp Thr Ile Thr Ser Lys Val Pro Thr Asp Arg Glu Asp Ser

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Gly Ala Arg Glu Ala Val Phe Ala Val Asn Ala Leu Asn Ile Ser Glu
65 70 75 80
Lys Gln His Glu Val Tyr Trp Lys Val Ser Glu Asp Lys Lys Ala Lys
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Cys Ala Glu Lys Gly Lys Ser Lys Gln Thr Glu Cys Leu Asn Tyr Ile
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Arg Val Leu Gln Pro Leu Ser Ala Thr Ser Leu Tyr Val Cys Gly Thr
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Phe Leu Gly Lys Asn Glu Asp Gly Lys Gly Arg Cys Pro Phe Asp Pro
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Thr Ser Tyr Asn Phe Leu Gly Ser Glu Pro Ile Ile Ser Arg Asn Ser
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Ser His Ser Pro Leu Arg Thr Glu Tyr Ala Ile Pro Trp Leu Asn Glu
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Pro Ser Phe Val Phe Ala Asp Val Ile Arg Lys Ser Pro Asp Ser Pro
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Asp Gly Glu Asp Asp Arg Val Tyr Phe Phe Phe Thr Glu Val Ser Val
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Cys Lys Gly Asp Gln Gly Gly Leu Arg Thr Leu Gln Lys Lys Trp Thr
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Ser Phe Leu Lys Ala Arg Leu Ile Cys Ser Arg Pro Asp Ser Gly Leu
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755 760 765

Ser Ala Leu Leu Ile Gly Lys Lys Lys Pro Lys Ser Asp Phe Cys Asp
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Arg Glu Gln Ser Leu Lys Glu Thr Leu Val Glu Pro Gly Ser Phe Ser
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Gln Gln Asn Gly Glu His Pro Lys Pro Ala Leu Asp Thr Gly Tyr Glu
805 810 815

Thr Glu Gln Asp Thr Ile Thr Ser Lys Val Pro Thr Asp Arg Glu Asp
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Ser Gln Arg Ile Asp Asp Leu Ser Ala Arg Asp Lys Pro Phe Asp Val
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Ser Asp Ser Ser Asp Thr Cys Cys Pro Cys Gly Trp Ile Pro Tyr Gln
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Glu Arg Cys Phe Tyr Ile Ser His Thr Leu Gly Ser Leu Glu Glu Ser
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Gln Lys Tyr Cys Thr Ser Leu Ser Ser Lys Leu Ala Ala Phe Asp Glu
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Leu Pro Ser Gly Leu Glu Glu Leu Leu Asp Arg Ser Lys Ser Tyr Trp
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Ala Val Gly Arg Ile Leu Pro Cys Arg Thr Thr Cys Leu Arg Tyr Leu
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[0051]

[Brief Explanation of the Drawings]

[Figure 1] It shows the binding property between CHO cells expressing CD72 and mCD100-Fc in example 1.

[Figure 2] It shows the IgG1 specific antibody production-promoting activity of CD100 in example 2.

[Figure 3] It shows the antibody production induction-promoting activity of CD100 in vivo in example 3.

[Docum[redacted]] Drawing

Fig.1

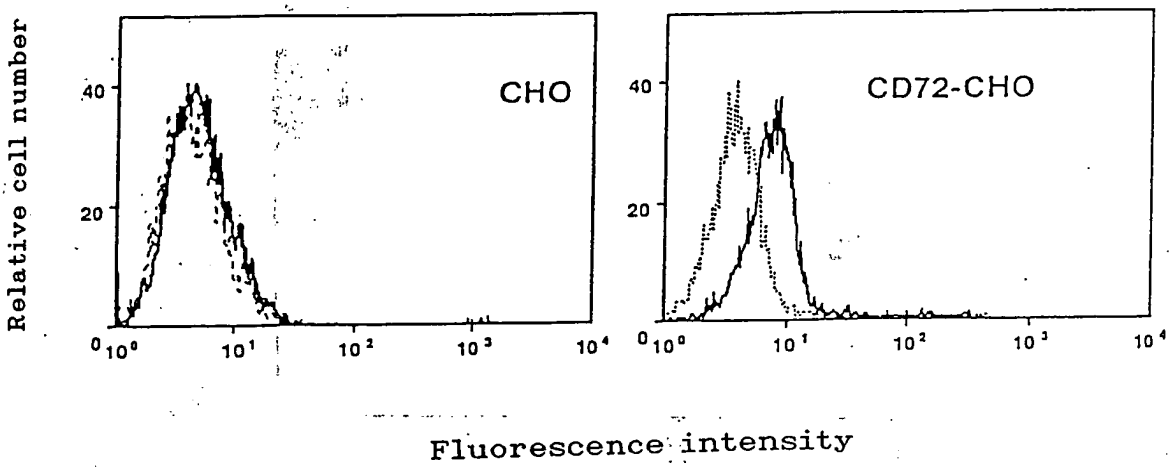


Fig.2

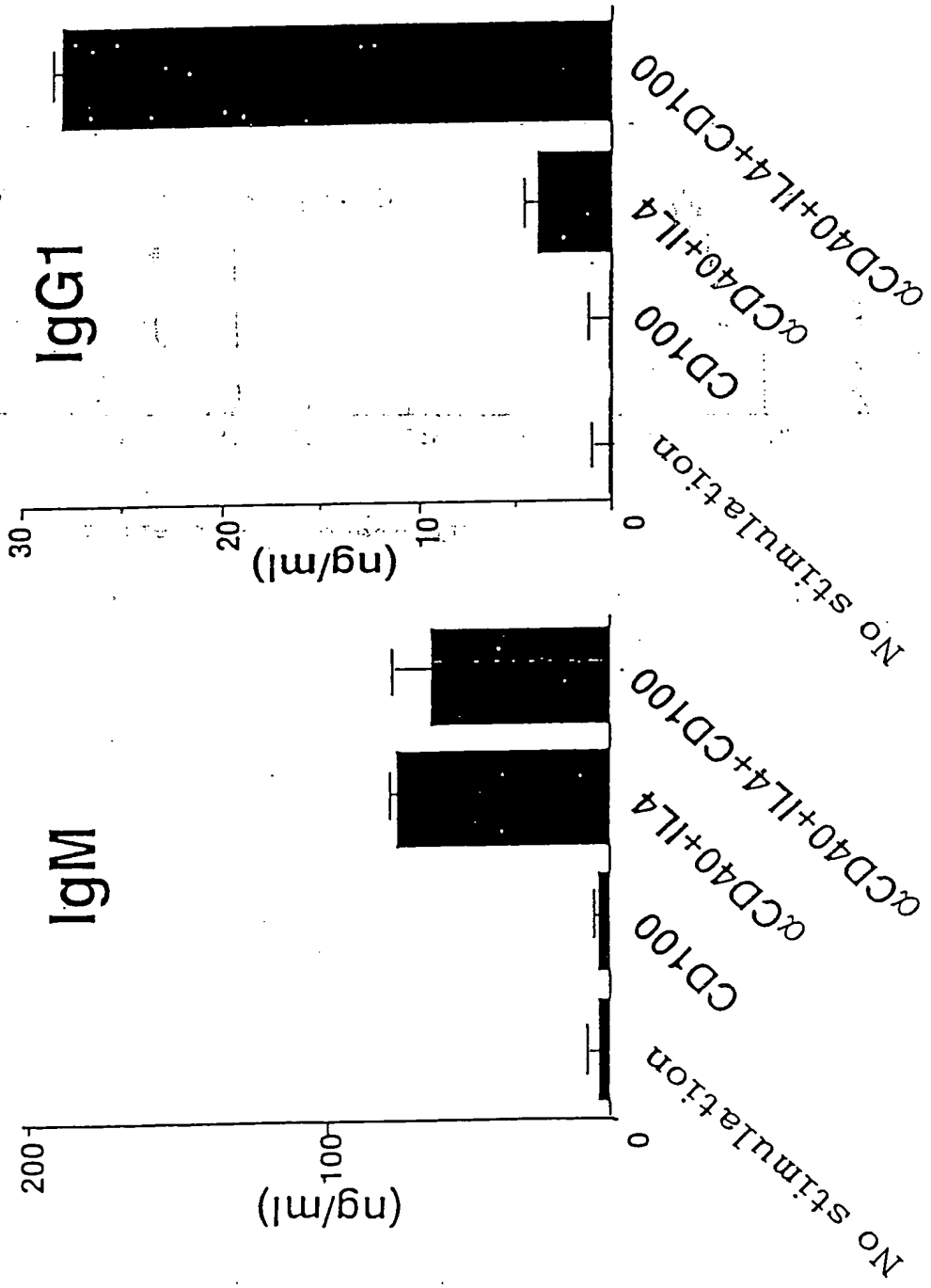
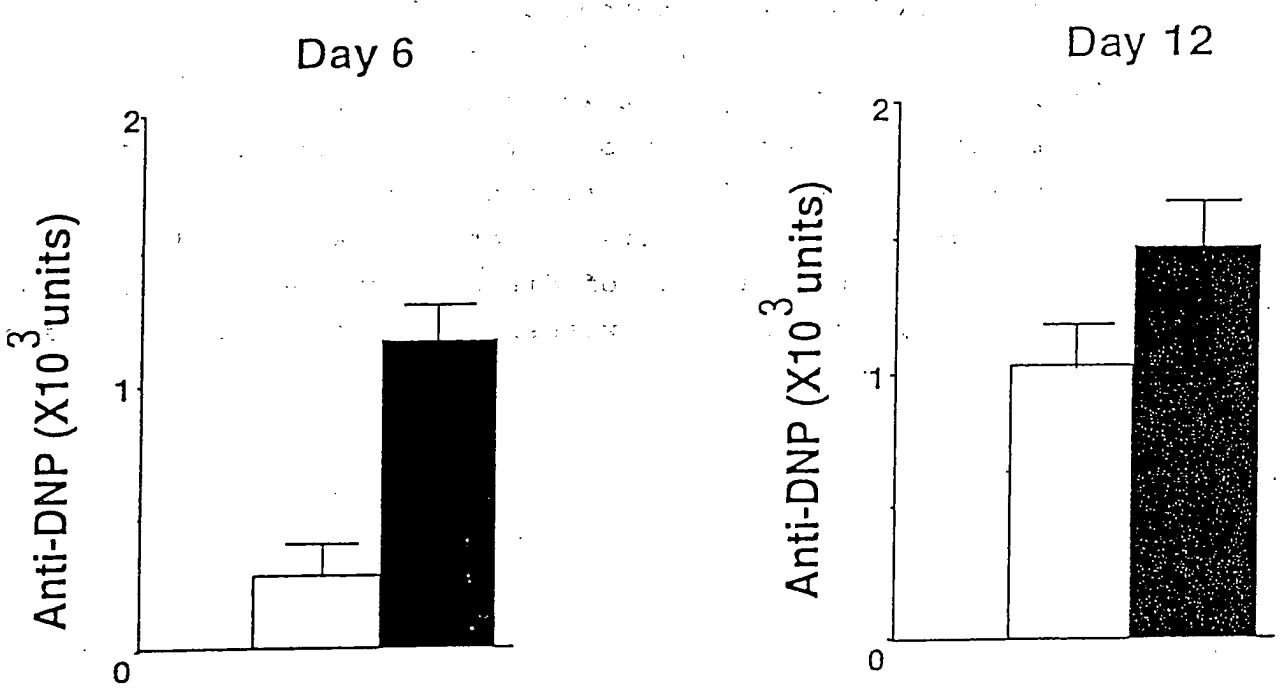

IgG1
IgM
(ng/ml)
0
10
20
30
100
200
No stimulation
CD100
αCD40+IL4
αCD40+IL4+CD100

Fig.3



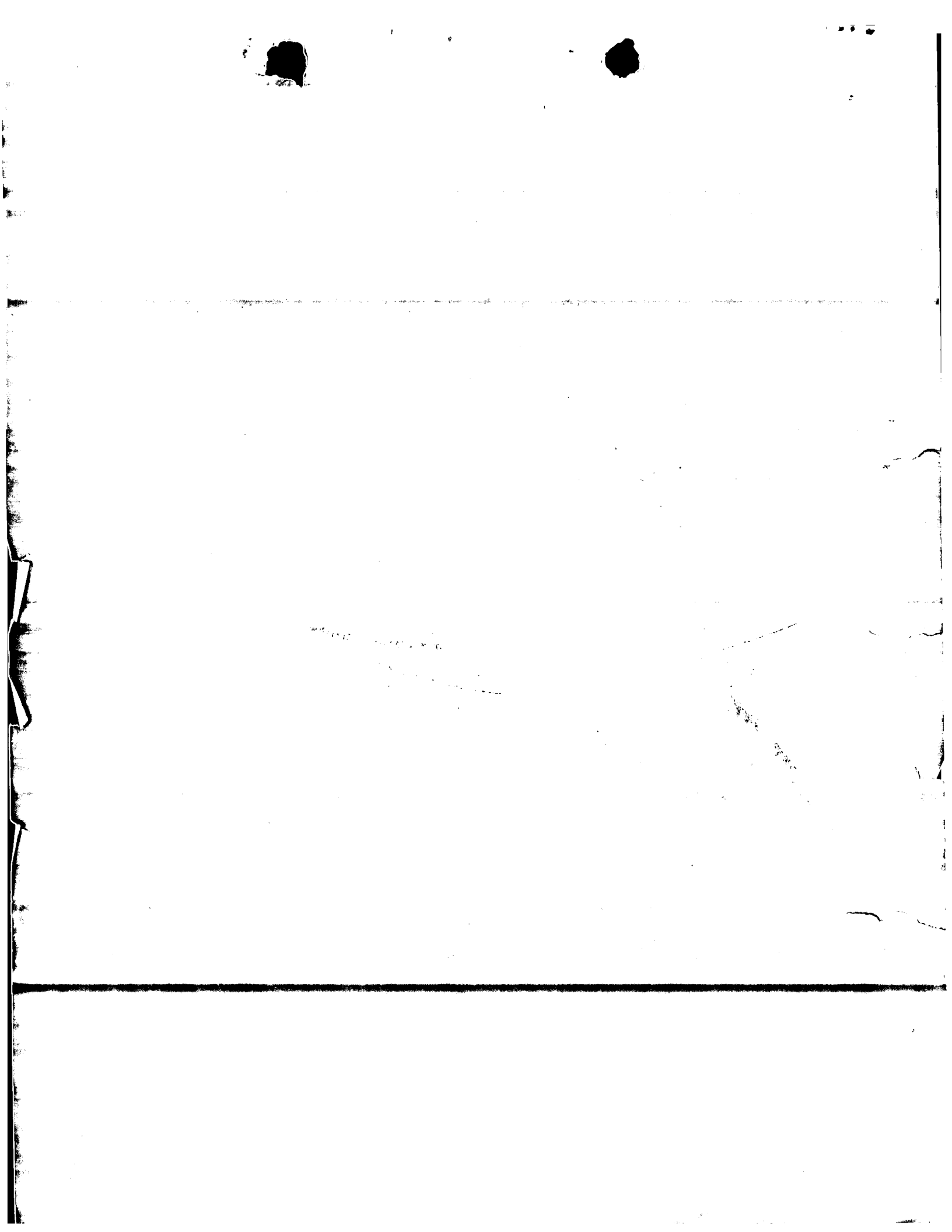
[Document] ABSTRACT

[Abstract]

[Problems] This invention provides superior screening methods.

[Means for solving problems] The present invention provides a method of screening a compound or salt thereof capable of changing the binding property between CD100 or salt thereof and CD72 or salt thereof, characterized by using CD100 or salt thereof and CD72 or salt thereof.

[Effects] This method is useful as a method of screening a CD72 agonist which is usable as drugs for prevention or treatment of viral infection or diseases, bacterial or fungal infection or diseases, cancer and the like, or a CD72 antagonist which is usable as drugs for prevention or treatment of diseases caused by abnormal antibody production or excessive antibody production and the like.



特許協力条約

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ＰＣＴ／ＪＰ００／０３５５８　　　ＲＯ１０５

ＰＣＴ

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〔ＰＣＴ規則２０．５（ｃ）〕

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出願人又は代理人の書類記号　２６１１ＷＯ０Ｐ	重　要　な　通　知	
国際出願番号 ＰＣＴ／ＪＰ００／０３５５８	国際出願日（日．月．年） ０１．０６．００	優先日（日．月．年） ０３．０６．９９
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様式ＰＣＴ／ＲＯ／１０５（１９９８年7月）